Research Modernisation Deal

Eine Strategie zur Modernisierung der Forschung und zum Ausstieg aus Tierversuchen

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Zusammenfassung

Erstaunliche Fortschritte in der Entwicklung von Technologien führen heutzutage zu grundlegenden Veränderungen in der biomedizinischen Forschung und bei regulatorischen Prüfverfahren.

Eine entsprechende Weiterentwicklung ist auch in den kommenden Jahren zu erwarten. Bislang stützte sich die Forschung auf die Verwendung von Tieren zur Abbildung menschlicher Krankheiten oder zur Vorhersage von Reaktionen des Menschen auf Medikamente oder andere Substanzen. Doch derzeit vollzieht sich ein Wandel hin zu Methoden, die auf der menschlichen Biologie basieren – ein Umbruch, der weltweit zu Veränderungen in Politik und Praxis führt. Bei Organisationen zur Forschungsförderung wächst das Bewusstsein, dass Tierversuche nicht dazu geeignet sind, die Wirksamkeit und das toxikologische Risiko von potenziellen Wirkstoffen zu ermitteln, und dass sie zudem die Entwicklung potenzieller Heilmittel behindern. In der heutigen Medikamentenentwicklung, die auf Tierversuchen basiert, versagen etwa 95 Prozent der neuen Medikamente in nachfolgenden klinischen Studien am Menschen. Zudem dauert die Markteinführung 10 bis 15 Jahre und verursacht Kosten in Höhe von mehr als 2 Mrd. Euro. Diese hohen Durchfallquoten lassen sich weder wirtschaftlich noch ethisch rechtfertigen. Bemühungen für eine grundlegende Veränderung der Forschungslandschaft sind daher dringend erforderlich.

Die folgenden wichtigen Punkte sollten berücksichtigt werden:

- Systematische Reviews, die in Fachzeitschriften veröffentlicht wurden, belegen die Einschränkungen bei der Übertragung von Ergebnissen aus Tierversuchsstudien auf die Behandlung von Menschen in zahlreichen Therapiebereichen. Weniger als 10 Prozent aller scheinbar vielversprechenden Ergebnisse aus der Grundlagenforschung werden innerhalb von 20 Jahren routinemäßig klinisch eingesetzt.
- Zwischen 50 und 89 Prozent der Ergebnisse aus der präklinischen Forschung sind nicht reproduzierbar, wobei Tierversuche einen ernstzunehmenden Problembereich darstellen.
- Bedeutende wissenschaftliche Erfolge in verschiedenen Therapiegebieten wie Diabetes und Brustkrebs stützen sich auf klinische Studien von menschlichen Krankheiten mit Erkrankten. Anhand von Tierversuchen wären diese Erfolge nicht möglich gewesen.

Es ist zunehmend erkennbar, dass sich Ergebnisse aus Tierversuchen nicht zuverlässig auf die medizinische Behandlung von Menschen oder anderen Tieren übertragen lassen. Daneben beobachten wir auch die fortschreitende Entwicklung und Implementierung von Alternativtechnologien, die Tierversuche ablösen. Doch vor allem wächst in unserer Gesellschaft ein Bewusstsein für das moralische Dilemma von Tierversuchen.
Öffentliche, private und gemeinnützige Fördergeber müssen ihre Budgets für Tierversuche kürzen und die Gelder stattdessen für tierfreie Methoden einsetzen. Um die Verwendung von Tieren in Versuchen zu beenden, empfehlen wir die Erarbeitung einer Strategie, die die folgenden entscheidenden Schritte umfasst:

1. **In Bereichen, in denen sich die Ergebnisse aus Tierversuchen nachweislich schlecht und unzuverlässig auf den Menschen übertragen lassen und in denen Tierversuche den Fortschritt behindern, sollte die Verwendung von Tieren unverzüglich eingestellt werden.**
2. **Mithilfe von kritischen wissenschaftlichen Untersuchungen sollten jene Bereiche ermittelt werden, in denen die Durchführung von Tierversuchen die menschliche Gesundheitsfürsorge bzw. den Umweltschutz nicht vorangebracht hat. Der Einsatz von Tieren in diesen Bereichen sollte daher schrittweise eingestellt werden.**
3. **Es sollten transparente, aussagekräftige prospektive und retrospektive Bewertungen gemäß der Richtlinie 2010/63/EU des Europäischen Parlaments und des Rates vom 22. September 2010 zum Schutz der für wissenschaftliche Zwecke verwendeten Tiere durchgeführt werden.**
4. **In weltweiter Zusammenarbeit mit Behörden und Einrichtungen sollte eine Harmonisierung und Förderung der internationalen Akzeptanz von tierversuchsfreien Verfahren zur Erfüllung der gesetzlichen Anforderungen an Toxizitätsprüfungen erfolgen.**
5. **Die finanzielle Förderung sollte umverteilt werden – von Tierversuchen hin zur Entwicklung tierfreier Test-verfahren.**
6. **Weiterbildung und Schulung von Forschenden und Behördenmitarbeitenden hinsichtlich der Vorteile und Anwendung tierfreier Methoden.**

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I. Einleitung

„Wenn man über Fortschritte in der Medizin liest, hat man oft den Eindruck, dass der lang erwartete Durchbruch bei Krebs, Alzheimer, Schlaganfall, Arthrose und unzähligen weniger verbreiteten Krankheiten direkt hinter der nächsten Ecke wartet. Aber es stellt sich heraus, dass wir in einer Welt mit sehr vielen Ecken leben.“[1]

Diese Feststellung des Wissenschaftsjournalisten und Bestsellerautors Richard Harris findet in den Herzen und Köpfen jedes Menschen Resonanz, der an einer unheilbaren Krankheit leidet oder eine Person kennt, die von einer solchen betroffen ist. Die US-amerikanischen National Institutes of Health (NIH), der weltweit größte Geldgeber für biomedizinische Forschung, berichtet, dass „die Medikamenten-Durchfallquote [bei neuen Arzneimitteln] in klinischen Studien am Menschen bei etwa 95 Prozent liegt“[2] – und das, obwohl diese Arzneimittel in präklinischen Tierversuchen für sicher und wirksam befunden wurden.

In der EU wird mit verschiedenen Ansätzen versucht, dieses Problem zu lösen. Auf mitgliedstaatlicher Ebene haben sowohl die Niederlande[3] als auch das Vereinigte Königreich[4] staatlich unterstützte Strategien zur Reduzierung und zum Ersatz von Tierversuchen entwickelt. Auf EU-Ebene setzt sich das EU-Referenzlabor für Alternativen zu Tierversuchen (EURL ECVAM), ein integraler Bestandteil der Gemeinsamen Forschungsstelle (Joint Research Centre, JRC) der Europäischen Kommission, dafür ein, Tierversuche sowohl in der biomedizinischen Forschung als auch in Toxizitätstests mit tierfreien Methoden zu ersetzen. So hat das EURL ECVAM beispielsweise eine Studie zur Überprüfung der Verwendung alternativer Methoden in der biomedizinischen Forschung in Auftrag gegeben. Das Referenzlabor wies darauf hin, dass „es daher wichtig ist, die Anwendung alternativer Methoden zu fördern, um die erhebliche Abhängigkeit von Tierversuchen bei der Durchführung von Forschungsarbeiten zu bekämpfen“. Ergänzend bemerkte das EURL ECVAM: „Alternativmethoden versprechen, die menschliche Physiologie effektiver nachbilden zu können als viele Tiermodelle. Die Umstellung auf neue tierfreie Methoden und Forschungsstrategien kann daher zu einem besseren Verständnis der humanspezifischen Biologie und von menschlichen Krankheiten führen.“[5]
Die Akzeptanz tierfreier Techniken in einer Region oder einem Land ebnet den Weg für die internationale Harmonisierung und weitere gesetzliche Abschaffung von Tierversuchen. Insbesondere in den letzten 20 Jahren wurden erhebliche Fortschritte bei der Entwicklung, Validierung, Implementierung und behördlichen Zulassung tierfreier Technologien für die Bewertung von menschlichen Gesundheitsendpunkten verzeichnet, darunter Hautreizung und -verätzung, schwere Augenschäden, Hautempfindlichkeit, Hautresorption und Phototoxizität. Daneben wurden auch als besonders grausam bekannte internationale Testrichtlinien abgeschafft, zum Beispiel Test Nr. 401 der Organisation für wirtschaftliche Zusammenarbeit und Entwicklung (OECD) – auch bekannt als LD50-Test. Es gibt heute Möglichkeiten, die Anwendung validierter, tierfreier Testmethoden für die regulatorische Bewertung zu verstärken und zu harmonisieren. Indem wir diese Verfahren anwenden, können wir im entsprechenden rechtlichen Rahmen einen besseren Schutz der menschlichen Gesundheit und der Umwelt gewährleisten.

Die Richtlinie 2010/63/EU des Europäischen Parlaments und des Rates vom 22. September 2010 zum Schutz der für wissenschaftliche Zwecke verwendeten Tiere soll gewährleisten, dass die Grund-sätze des 3R-Prinzips – Replace (Vermeiden), Reduce (Verringern) und Refine (Verbessern) – bei der Durchführung von Tierversuchen in[6]nerhalb des rechtlichen Rahmens zur Anwendung kommen. Die Richtlinie erkennt letztlich an, dass das endgültige Ziel darin besteht, alle wissenschaftlichen Verfahren, bei denen Tiere eingesetzt werden, zu ersetzen – sowohl für die biomedizinische Grundlagenforschung als auch zur Erfüllung regulatorischer Anforderungen.[7]

Vor dem Hintergrund der Europäischen Bürgerinitiative „Save Cruelty Free Cosmetics – Für ein Europa ohne Tierversuche“[8] und der Entschließung des Europäischen Parlaments aus dem Jahr 2021 zur „Beschleunigung eines Übergangs zu Innovationen ohne die Verwendung von Tieren in der Forschung, bei vorgeschriebenen Versuchen und in der Bildung“[9] ist es von entscheidender Bedeutung, dass die EU mit den wissenschaftlichen Entwicklungen Schritt hält. Moderne Richtlinien müssen das Ziel widerspiegeln, Tierversuche langfristig zu beenden, und gleichzeitig die Entwicklung und Anwendung innovativer, tierfreier Methoden unterstützen, die auf humanbiologischen Grundlagen beruhen. Zur

Verwirklichung dieses Ziels stellen wir mit diesem Bericht ein Konzept für die Ablösung von Tierversuchen vor. Wir benennen strategische Prioritäten und ergänzen diese mit weiteren Informationen zu Bereichen der regulatorisch vorgeschriebenen (gesetzlich erforderlichen) und nicht-regulatorisch vorgeschriebenen Forschung hinzu, in denen die Durchführung von Tierversuchen unverzüglich bzw. in naher Zukunft ersetzt werden könnte. Der Bericht enthält zudem Informationen zu Bereichen, in denen die Weiterentwicklung, Validierung und Implementierung von tierfreien Testmethoden erforderlich ist.

II. Eingeschränkte Voraussagefähigkeit von Tierversuchen in der Forschung

Zahlreiche wissenschaftliche Untersuchungen belegen, dass Tierversuche fehlerhaft sind und darüber hinaus anderen Testmethoden, die auf dem Weg zur Heilung menschlicher Krankheiten besser geeignet sind, sowohl finanzielle als auch intellektuelle Ressourcen vorenthalten. Die Tatsache, dass Tierversuche keine zuverlässige Vorhersage über die Wirkung einer Substanz beim Menschen erlauben, beruht auf verschiedenen Faktoren. Dazu gehören unter anderem eine verzerrte Darstellung der Datenlage bei der Berichterstattung und Veröffentlichung, ein undurchdachtes Studiendesign und eine unzureichende Stichprobengröße.[10] Der entscheidende Faktor ist jedoch die Tatsache, dass die Ergebnisse aus Tierversuchen aufgrund von immanenten biologischen und genetischen Unterschieden schwer auf den Menschen übertragen werden können – selbst mit einem optimal kontrollierten und bestmöglich durchgeführten Studiendesign.

Fehlende Aussagekraft

Probleme mit der Reproduzierbarkeit (interne Validität) und der Übertragbarkeit (externe Validität) tragen dazu bei, dass sich Erkenntnisse aus der biomedizinischen Forschung, die mittels Tierversuchen gewonnen wurden, nicht aus dem Forschungslabor in die klinische Anwendung an Erkrankten übertragen lassen. Die interne Validität von Tierversuchen wird durch ein schlechtes Studiendesign beeinträchtigt, beispielsweise wenn Personen, die Tierversuche leiten, keine Maßnahmen zur Vermeidung von Voreingenommenheit treffen. Dazu gehört es beispielsweise, sicherzustellen, dass Personen, die Versuche durchführen oder Daten analysieren, nicht wissen, ob die Tiere oder die Proben zur Behandlungs- oder zur Kontrollgruppe gehören (Verblindung). Nach einer Meta-Analyse systematischer Reviews vorklinischer Tierversuche in verschiedensten Therapiebereichen stellten Forschende der Universität Oxford fest, dass der Nutzen von untersuchten Behandlungsmethoden aufgrund fehlender Maßnahmen zur Verringerung der Ergebnisverzerrung aus Tierversuchen wahrscheinlich überschätzt wird. Dies kann das Vertrauen in die Ergebnisse verringern und gleichzeitig begrenzte Ressourcen verschwenden.[11] Sie schlussfolgerten: „Verzerrte Ergebnisse aus der tierexperimentellen Forschung liefern mit geringerer Wahrscheinlichkeit vertrauenswürdige Ergebnisse oder stichhaltige Gründe für eine Forschung, die dem Menschen zugutekommt. Daneben verursachen sie eine Verschwendung von knappen Ressourcen.“[11] Die Forschenden sprachen zudem folgende Empfehlung aus: „Studien am Menschen werden häufig auf der Grundlage der Ergebnisse aus Tierversuchen gerechtfertigt. Unsere Ergebnisse lassen darauf schließen, dass Tierversuche, deren Resultate unangemessen verzerrt wurden, nicht Teil der Begründung für klinische Studien am Menschen sein sollten.“[11]

Eine Untersuchung aus dem Jahr 2015 ergab, dass zwischen 50 und 89 Prozent der präklinischen Forschung, die zu einem großen Teil Tierversuche umfasst, nicht reproduziert werden konnte.

Eine schlechte interne Validität führt dazu, dass viele Tierversuche nicht reproduziert werden können. Dies ist jedoch ein zentraler Aspekt des wissenschaftlichen Prozesses, der auf die potenzielle Validität von Ergebnissen hinweist. Es ist daher nicht verwunderlich, dass eine Untersuchung aus dem Jahr 2015 ergab, dass zwischen 50 und 89 Prozent der präklinischen Forschung, die zu einem großen Teil Tierversuche umfasst, nicht reproduziert werden konnte.[12]

Die Defizite von Tierversuchen lassen sich jedoch nicht einfach durch eine Verbesserung des Studiendesigns beheben, denn mit Tierversuchen kann niemals eine externe Validität erreicht werden. Externe Validität bezeichnet das „Ausmaß, in dem sich Forschungsergebnisse aus einem Setting, einer Population oder einer Art zuverlässig auf andere Settings, Populationen und Arten übertragen lassen“.[13]

Aufgrund inhärenter Unterschiede zwischen Mensch und Tier können nichtmenschliche Tiere nicht als Analoga dienen, um die spezifischen biologischen Wirkungen zu verstehen, die Arzneimittel und Chemikalien auf den Menschen haben. Laut Wall und Shani können selbst „extrapolierte Ergebnisse von Studien mit zig Millionen Tieren die Reaktion beim Menschen nicht genau vorhersagen“.[14] In einem Review im Journal of Translational Medicine aus dem Jahr 2018 bezeichnen Pandora Pound und Merel Ritskes-Hoitinga die Speziesunterschiede als unüberwindbares Problem für die externe Validität präklinischer Tiermodelle.[13] Versuche, die Speziesunterschiede zu kontrollieren oder zu korrigieren, würden zu einem „Extrapolator’s Circle“ (Extra-polationskreis) führen: „Wenn wir feststellen wollen, ob die Wirkungsweise einer Substanz bei Tieren der Wirkungsweise der Substanz beim Menschen hinreichend ähnlich ist, um eine Extrapolation zu rechtfertigen, müssen wir die entsprechende Wirkungsweise beim Menschen kennen. Und wenn wir die Wirkungsweise beim Menschen bereits kennen, dann dürfte der anfängliche Tierversuch überflüssig gewesen sein.“ Weiterhin befassen sich Pound et al. auch mit der besorgniserregenden Entwicklung unter Personen, die an Tierversuchen beteiligt sind, die Frage des Spezies-unterschieds und die Auswirkungen auf die externe Validität zu verharmlosen – ein Problem, das jedoch von einer Reihe von Forschenden durchaus anerkannt wird.[15,16] Wie Pound und Ritskes-Hoitinga weiter ausführen, ist es nicht verwunderlich, dass die Frage des Speziesunterschieds heruntergespielt wird, da sich die Experimentierenden ansonsten mit der „Möglichkeit auseinandersetzen müssten, dass das präklinische tierexperimentelle Forschungsparadigma nicht mehr viel zu bieten hat“.[13] Es besteht ein wachsender wissenschaftlicher Konsens darüber, dass weit mehr erreicht werden kann, wenn humanrelevante Forschungsmethoden und -technologien angewendet werden, um Fragen im Bereich der Humanbiomedizin und Umweltforschung oder im Rahmen regulatorischer Bewertungsparadigmen zu lösen. Wie eine kürzlich veröffentlichte Branchenstudie hervorhob, ist es an der Zeit, die Entdeckung von Arzneimitteln und die Toxikologie zu humanisieren.[17] Dies ist besonders relevant in Deutschland, dem größten Biotechnologie-Markt nach den USA.[18]

Mangelnde Übertragbarkeit

Angesichts des Problems der schlechten Validität und Reproduzierbarkeit von Tierversuchen ist es nicht verwunderlich, dass sich die Ergebnisse aus Tierversuchen häufig nicht klinisch relevant auf menschliche Erkrankte übertragen lassen. Wie bereits erwähnt, versagen laut den NIH neue Medikamente „in rund 95 Prozent der Studien am Menschen“[19] – obgleich sie in präklinischen Tierversuchen für sicher und wirksam befunden wurden.

John Ioannidis, Professor für Medizin, Gesundheitsforschung und Gesundheitspolitik an der US-amerikanischen Universität Stanford, wollte beurteilen, ob die biomedizinische Grundlagenforschung ihre Versprechen erfüllt oder nicht. Hierzu ermittelte er gemeinsam mit seinem Kollegium

Weniger als 10 Prozent aller scheinbar vielversprechenden Ergebnisse aus der Grundlagenforschung werden innerhalb von 20 Jahren routinemäßig klinisch eingesetzt.

101 Artikel, die in den renommiertesten medizinischen Fachzeitschriften veröffentlicht wurden und in denen ausdrücklich erklärt wurde, dass die Forschung zu neuen Anwendungsgebieten mit realistischem Potenzial für einen klinischen Durchbruch führen würde. Der Großteil der analysierten Artikel (63 Prozent) bezog sich auf Tierversuche. Die Untersuchungen von Professor Ioannidis und seinem Kollegium hinsichtlich der Übertragung der

Grundlagenforschung auf die klinische Anwendung ergab, dass weniger als 10 Prozent aller vielversprechenden Ergebnisse aus der Grundlagenforschung innerhalb von 20 Jahren routinemäßig klinisch eingesetzt werden.[20]

Eine in der medizinischen wissenschaftlichen Fachzeitschrift The BMJ veröffentlichte beeindruckende Analyse aus dem Jahr 2014 hat ergeben, dass Tierversuche entgegen der öffentlichen Wahrnehmung die Erkenntnisse auf dem Gebiet der menschlichen Gesundheit nicht vertieft oder zur Entwicklung von Behandlungen für menschliche Krankheiten geführt haben.[21] Die Schlussfolgerung der Studie lautet: „Wenn die tierexperimentelle Forschung auch künftig die beim Menschen zu erwartende Wirkung nicht zuverlässig prognostizieren kann, dann erscheint die weitere öffentliche Billigung und Finanzierung der präklinischen Forschung an Tieren unangebracht."[21]

Die Schwierigkeiten bei der Übertragung von Ergebnissen aus Tierversuchen auf menschliche Erkrankte werden durch die Gefangenhaltung der Tiere und die unnatürlichen Bedingungen im Versuchslabor weiter verschärft, da diese das natürliche Verhalten der Tiere beeinträchtigen.[22] Das entbehrungsreiche Leben im Versuchslabor erhöht den Stresslevel der Tiere. Aufgrund der hierdurch veränderten Physiologie und Neurobiologie weisen die Tiere verschiedene Formen von Psychosen und Psychopathien auf.[23–27] Darüber hinaus sind

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Tiere, die in Versuchslaboren ihre Physiologie und die Neurobiologie verändert haben, keine guten „Modelle" für ihre Artgenossen in der freien Natur.

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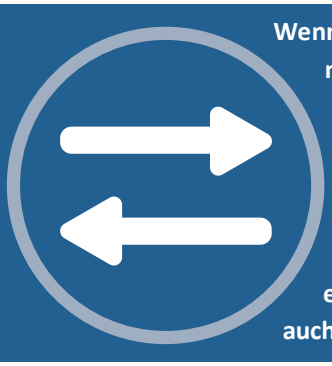
Beleg 1: Mangel an klinischem Erfolg

Die Erfolglosigkeit grundlegender und angewandter wissenschaftlicher tierexperimenteller Studien zeigt sich vielleicht am deutlichsten in den zahllosen scheinbar vielversprechenden Behandlungen, die beim Menschen einfach nicht geholfen haben. Schlaganfallstudien mit Tieren beispielsweise waren ein völliger Misserfolg. Forschende des Instituts für Schlaganfall- und Demenzforschung in München haben die Defizite wie folgt beschrieben:

> „In Nagetiermodellen wurden mehr als 1.000 neuroprotektive Verbindungen getestet, um das Schlaganfallergebnis zu verbessern. […] Viele Wirkstoffe reduzierten tatsächlich die Schädigung des Gehirns (in den meisten Fällen gemessen als verringertes Infarktvolumen) in experimentellen Schlaganfall-Modellen mit Nagetieren. Von diesen wurden etwa 50 Neuroprotektiva in mehr als 100 klinischen Schlaganfallstudien getestet, doch keiner der Wirkstoffe hat das Outcome bei klinischen Schlaganfallerkrankten verbessert."[29]

Onkologische Medikamente, die ebenfalls in Tierversuchen getestet werden, weisen eine Erfolgsquote von nur 3,4 Prozent auf.[30] Dieses Problem tritt in vielen menschlichen Krankheitsbereichen auf. Eine Fülle an Literatur dokumentiert den Misserfolg verschiedener Tiermodelle bei neurodegenerativen Erkrankungen, wie etwa Alzheimer, bei denen die Ausfallrate für neue Arzneimittel in der klinischen Phase bei 99,6 Prozent liegt.[31]

III. Die Notwendigkeit eines Paradigmenwechsels



Wenn wir unsere begrenzten öffentlichen Mittel verantwortungsvoll einsetzen wollen, dann müssen wir eine Forschung fördern, die zu einer erfolgreichen Behandlung des Menschen führt – sei es Grundlagenforschung oder angewandte Forschung. Doch obwohl alles darauf hindeutet, dass die Entwicklung neuer Behandlungen und Heilmittel für menschliche Krankheiten durch eine Grundlagen- und angewandte Forschung mithilfe von Tierversuchen erschwert wird, hat diese Erkenntnis bislang keine ausreichende Überprüfung der Prioritäten bezüglich Forschung und Förderung durch nationale und europäische Behörden zur Folge. Ein solcher Paradigmenwechsel ist sowohl innerhalb als auch außerhalb der EU von entscheidender Bedeutung.

Einige Mitglieder der wissenschaftlichen Gemeinschaft haben begonnen, sich für Veränderungen einzusetzen. So unterstützten beispielsweise 15 Forschende der US-amerikanischen Vanderbilt University den Einsatz eines evidenzbasierten Ansatzes, mit dem sich die Entwicklung nützlicher Medikamente für Erkrankte, die diese benötigen, beschleunigen lassen. Hierzu veröffentlichten sie einen Artikel aus dem Jahr 2017, der die Abschaffung von Tierversuchen fordert, wenn eindeutige Beweise dafür vorliegen, dass die „Tiermodelle“ nicht nützlich oder aussagekräftig im Hinblick auf menschliche Krankheiten sind:

„In der Literatur finden sich zahlreiche Beispiele für Widersprüche und Unstimmigkeiten bezüglich der Wirkungsweise von Substanzen bei Tieren und Menschen. Dazu gehören auch viele Fälle, in denen erfolgsversprechende Ergebnisse aus Tierversuchen nicht zu einer klinisch signifikanten Wirksamkeit beim Menschen führten. Dies gilt insbesondere für einige Behandlungsgebiete wie neurodegenerative, psychische Krankheiten und Erkrankungen des Zentralnervensystems sowie Sepsis- und entzündliche Erkrankungen.

Die Komplexität der translationalen Forschung stellt eine bedeutende Chance zur Erforschung neuer Ansätze dar, die in erfolgreicher und effizienter Weise Ergebnisse hervorbringen, welche dem menschlichen Nutzen möglichst nahe kommen. Gestützt auf einige anschauliche Beispiele, denen wir in unserem ‚Drug Repurposing Program‘ (Programm zur Neuorientierung bezüglich Arzneimittel) begegneten, möchten wir hiermit einen Ansatz zur Diskussion stellen. Dieses Konzept dient der Beurteilung dessen, wenn es angebracht ist, den ‚letzten Versuch zuerst‘ durchzuführen, d. h. direkt mit Studien am Menschen fortzufahren, wenn es wahrscheinlich ist, dass Tierversuche keine angemessenen Daten liefern, die sich auf Anwendungen von Interesse für den Menschen übertragen lassen. Dies stellt ein erhebliches – aber unserer Meinung nach vermeidbares – Hindernis bei der Einführung von Arzneimitteln dar.“[32]

Die Abkehr des allgemeinen Konsens von Tierversuchen lässt sich in verschiedenen Bereichen beobachten, beispielsweise in Publikationen zur beschränkten Aussagekraft von Tierversuchen[21], in einer verstärkten Sensibilisierung der Gesellschaft für die kognitiven Fähigkeiten und die Empfindungsfähigkeit von Tieren[33] und in der rasant schwindenden öffentlichen Akzeptanz von Tierversuchen.[34] Die Fachzeitschrift der Türkischen Gesellschaft für Gastroenterologie, Turkish Journal of Gastroenterology, hat die Veröffentlichung von Studien, in denen Tierversuche durchgeführt wurden, offiziell von ihren Websites verbannt. Der Herausgeber der Zeitschrift, Dr. Hakan Şentürk, schrieb, dass die neue Regelung die „wachsende Besorgnis über die mangelnde Übertragbarkeit der tierexperimentellen Forschung auf den Menschen zum Ausdruck bringt“.[35] Weiterhin erklärte er: „Wenn wir erkennen, dass die Abhängigkeit von grundsätzlich unzulänglichen Tiermodellen menschlicher Krankheiten in hohem Maße für klinisches Versagen verantwortlich ist, dann ist es nicht sinnvoll, diese Praxis weiter zu fördern. […] Stattdessen sollten humanrelevante Ansätze intensiver entwickelt und genutzt werden.“[35]

Bezeichnenderweise wird eine Abkehr von der tierexperimentellen Forschung zu einem erheblichen Wachstum des Wissenschafts- und Technologiesektors und zu einer schnelleren Amortisation von Investitionen in der Arzneimittelforschung und -entwicklung führen. Dies hat sich zum Beispiel nach dem Verbot von Tierversuchen für Kosmetika in der EU gezeigt, obwohl es anfänglich auch Widerstand bei Teilen der Industrie gab.[36] Wenn die Forschungsfinanzierung ihre Prioritäten hin zu humanrelevanten Versuchsmethoden verlagert und Methoden bevorzugt, die die menschliche Physiologie und Biologie nachbilden, ohne Tiere oder deren Gewebe zu verwenden, können Erkrankte benötigte Behandlungen sicherer und wahrscheinlich in kürzerer Zeit erhalten.[37] Da die staatliche Förderung von Forschungsaktivitäten begrenzt ist, erschwert die Abhängigkeit von Tierversuchen eine Forschung, die mit größerer Wahrscheinlichkeit wirksame Medikamente und Heilmittel hervorbringt.

IV. Chancen für wirtschaftlichen Fortschritt

Die hohen Kosten der Arzneimittelentwicklung

Mit einer Anordnung zur Abkehr von Tierversuchen und zum Einsatz fortschrittlicher wissenschaftlicher Methoden hat die EU die Möglichkeit, das Beschäftigungswachstum in den Bereichen Wissenschaft und Technologie rasant zu steigern und die Kosten des Gesundheitswesens für die Bevölkerung zu senken. Wie Meigs et al. in ihrem kürzlich erschienenen Review „Animal Testing and Its Alternatives – the Most Important Omics Is Economics" ausführen, hat sich eine „Ökonomie alternativer Ansätze entwickelt, welche die klassischen Tierversuche übertreffen".[36]

Auch die britische Fördergesellschaft Innovate UK bezeichnet tierfreie Technologien „als eine von mehreren neuen Technologien, die über das Potenzial verfügen, das künftige Wirtschaftswachstum in Großbritannien voranzutreiben". Die Agentur empfahl, britische Unternehmen in die Lage zu versetzen, diese „neuen Geschäftsmöglichkeiten" nutzen zu können.[38]

Die Markteinführung eines neuen Arzneimittels kann über 2 Mrd. Euro kosten und bis zu 15 Jahre dauern.[19] Ein Faktor für die hohen Forschungs- und Entwicklungskosten ist das beträchtliche Risiko, ein Produkt zu entwickeln, das niemals zu einem marktfähigen Medikament wird, weil es in klinischen Studien durchfällt. 95 Prozent aller Medikamente, die in Tierversuchen für sicher und wirksam befunden wurden, versagen beim Menschen[19], weil entweder unerwünschte Nebenwirkungen auftreten oder keine Wirksamkeit gegeben ist. Kacey Ronaldson-Bouchard und Gordana Vunjak-Novakovic, Forschende an der US-amerikanischen Columbia University, unterstützen die *In-vitro*-Forschung an menschlichem Gewebe bei der Arzneimittelentwicklung. Sie machten die folgenden Beobachtungen:

„Ebenso schädlich ist die vorsorgliche Eliminierung potenziell kurativer neuer Medikamente, da sich deren schädliche Auswirkungen bei Tieren nicht unbedingt auf den Menschen übertragen lassen. Diese falsch-positiven und falsch-negativen Ergebnisse stellen eine enorme finanzielle Belastung dar und führen zu Entscheidungen, bei denen die potenzielle Rentabilität eines Medikaments gegen die potenziellen Risiken abgewogen wird und nicht gegen das Potenzial des Medikaments, den Behandlungserfolg der Krankheit zu verbessern."[39]

Das Problem einer wirksamen und effizienten Markteinführung neuer Arzneimittel wird durch die mangelnde Reproduzierbarkeit präklinischer Studien noch verstärkt. Eine kürzlich vom Komitee für Wissenschaft und Technologie (Science and Technology Committee) des britischen Unterhauses durchgeführte Untersuchung der wissenschaftlichen Integrität staatlich finanzierter Forschungsaktivitäten unterstrich die aktuelle „Reproduzierbarkeitskrise" und wies auf die steigende Tendenz bezüglich Fehlverhaltens und Fehlern bei der Veröffentlichung hin.[40] Auch im Deutschlandfunk[41] und im Laborjournal[42] wurde darüber diskutiert, dass Voreingenommenheit und schlechte Statistik zu falsch-positiven Ergebnissen in wissenschaftlichen Publikationen führen und dass der Großteil der präklinischen Daten nicht reproduzierbar ist. Laut der konservativsten US-amerikanischen Schätzung führt das häufige Unvermögen, präklinische Forschungsergebnisse zu reproduzieren, zu jährlichen Ausgaben in Höhe von ca. 25 Mrd. Euro für irreführende Experimente.[12] Darüber hinaus werden auch in Fachzeitschriften, welche die ARRIVE-Richtlinien (Animal Research: Reporting of In Vivo Experiments)[43] unterstützen, immer wieder Studien veröffentlicht, die eine geringe Reproduzierbarkeit, ein schlechtes Preis-Leistungs-Verhältnis und eine Verschwendung von Tierleben belegen. Die ARRIVE-Richtlinien dienen dazu, die Berichterstattung über Tierversuche zu verbessern.[44]

Durch die Verwendung von humanrelevanten Technologien anstelle von teuren, zeitaufwendigen Tierversuchen mit ungenauen Ergebnissen könnten sich die Kosten für die Entwicklung neuer Medikamente drastisch senken lassen. In der Fachzeitschrift der American Society for Clinical Pharmacology and Therapeutics (ASCPT) äußerten sich Tal Burt et al. wie folgt:

„Die steigenden Kosten der Arzneimittelentwicklung verbunden mit ethischen Bedenken hinsichtlich der Risiken, Menschen und Tiere neuen chemischen Substanzen auszusetzen, führen zu einer bevorzugten Anwendung von klinischen Studien mit begrenzter Exposition, wie Microdosing-Studien oder andere Phase-0-Studien. Die Forschung unterstützt in zunehmendem Maß die Gültigkeit der Extrapolation von Erkenntnissen, die durch begrenzte Medikamentenexposition mit dem Phase-0-Ansatz gewonnenen werden, hin zur vollständigen therapeutischen Exposition. Eine zunehmende Anzahl von Anwendungsbereichen und Designoptionen zeigt die Vielseitigkeit und Flexibilität, die diese Ansätze Arzneimittelentwicklern bieten."[45]

Um ein Höchstmaß an Genauigkeit, Reproduzierbarkeit und Relevanz bei der Erforschung menschlicher Krankheiten zu erreichen, ist es unerlässlich, dass beträchtliche finanzielle Fördermittel für die Implementierung und weitere Erforschung zuverlässiger, humaner *In-vitro*- und *In-silico*-Konzepte zur Verfügung gestellt werden.

Beleg 2: Das Risiko irreführender Ergebnisse

Viele neuartige Medikamente scheitern in der klinischen Prüfung am Menschen, was einen enormen Zeit- und Investitionsverlust bedeutet. Darüber hinaus können sie Menschen auch Schaden zufügen. Im Jahr 2016 entwickelte ein portugiesischer Pharmahersteller ein Medikament, das bei Stimmungsschwankungen, Angst und motorischen Problemen aufgrund von neurodegenerativen Erkrankungen helfen sollte. Das Medikament wurde freiwilligen Teilnehmenden im Rahmen der klinischen Phase-I-Studie eines französischen Auftragsforschungsinstituts oral verabreicht. Sechs männliche Testpersonen im Alter von 28 bis 49 Jahren litten an starken Nebenwirkungen und mussten ins Krankenhaus eingeliefert werden. Eine Testperson wurde für hirntot erklärt und verstarb später. Wie ein Bericht über diesen Vorfall aufdeckte, wurde „bei den Tieren trotz einer 400-mal höheren Dosis als bei den menschlichen Testpersonen keine schädliche Wirkung festgestellt“.[46]

In seinem Artikel „TGN1412: From Discovery to Disaster“ aus dem Jahr 2010 berichtet Husain Attarwala von der US-amerikanischen Northeastern University über das tragische Ergebnis der 2006 durchgeführten klinischen Studie mit Theralizumab, einem immunmodulatorischen Medikament. Attarwala schrieb: „Nach [der] ersten Infusion einer Dosis, die 500-mal geringer war als die im Tierversuch als sicher eingestufte, befanden sich alle sechs Teilnehmenden in lebensbedrohlichem Zustand. Da ein Multiorganversagen drohte, wurden sie auf die Intensivstation verlegt.“[47] Fünf der sechs Teilnehmenden mussten nach der Anfangsdosis drei Monate im Krankenhaus bleiben, die sechste Testperson lag im Koma. Selbst ein halbes Jahr später litten die Teilnehmenden noch unter Kopfschmerzen und Gedächtnisverlust. Einem der Erkrankten mussten infolge einer Gewebs-Nekrose Zehen und Finger amputiert werden.[48] Attarwala schloss aus diesen und anderen Studien: „Arzneimittel, die in präklinischen Tiermodellen als sicher und wirksam eingestuft werden, können bei der Anwendung am Menschen sehr unterschiedliche pharmakologische Eigenschaften aufweisen.“[47]

Doch auch das Gegenteil ist der Fall: Heilverfahren, die bei Tieren nicht wirksam waren, blieben ungenutzt und Erkrankte warteten somit vergeblich auf lebensrettende Behandlungen. Penicillin beispielsweise wurde 1929 erstmals an Kaninchen getestet, doch da der Wirkstoff bei dieser Tierart keine offensichtliche Wirkung zeigte, blieb er mehr als zehn Jahre lang unbeachtet – was unzählige Menschenleben kostete. Die ersten klinischen Versuche am Menschen wurden erst in den 1940er-Jahren durchgeführt.[49] Forschende erklärten später, dass Penicillin zum Glück nicht zuerst an Meerschweinchen getestet wurde, denn bei diesen Tieren wirkt das Antibiotikum tödlich. Bei einem solchen Ergebnis im Tierversuch wäre Penicillin möglicherweise nie am Menschen getestet worden.[50]

Beschäftigungs- und Wirtschaftswachstum im Technologiesektor

Der Markt für humanbasierte *In-vitro*-Technologie für die biomedizinische Forschung und für Versuche wächst rasant. BCC Research schätzt, dass der Markt für zellbasierte Tests bis 2027 auf 44,3 Mrd. Euro anwachsen wird, und dass der Markt für induzierte pluripotente Stammzellen (iPSCs) im Jahr 2026 ein Volumen von 4,1 Mrd. Euro erreichen wird.[51,52] Weitere Marktforschung geht zudem davon aus, dass der weltweite Markt für Organ-Chip-Technologie bis 2028 ein Volumen von ca. 763,8 Millionen Euro umfassen wird.[53]

Die Anwendung dieser neuen Technologien nimmt in unterschiedlichen Sektoren immer weiter zu. Während deutsche Kosmetikfirmen wie Beiersdorf aufgrund des Tierversuchsverbots schon seit Jahren auf tierfreie Methoden setzen und diese selbst entwickeln[54], lassen auch Firmen wie Merck und Bayer verlauten, dass sie aus Tierversuchen aussteigen wollen und stattdessen auf neue Technologien wie Organ-Chips setzen.[55–57] Große Chemiekonzerne wie BASF investieren schon seit Jahren in die Entwicklung eigener tierfreier Methoden: Seit 2004 werden hierfür jährlich siebenstellige Beträge ausgegeben, 2020 beispielsweise waren dies 3,5 Millionen Euro.[58]

Auch in der Forschung besteht ein erhöhtes Interesse an tierfreien Technologien. In Berlin wurde aus den Reihen der Berliner TU und der Charité Berlin im Jahr 2018 zudem der Bau eines neuen Forschungsgebäudes beantragt und nach erfolgreicher Verteidigung vor dem Wissenschaftsrat auch genehmigt. Der 34 Millionen Euro teure Bau mit dem Namen „Der Simulierte Mensch“ (Si-M) zielt darauf ab, dass Mitarbeitende aus beiden Institutionen hier gemeinsam „Funktionen menschlicher Zellen und Gewebe mit neuen Technologien der 3D-Kultivierung, der Multi-Organ-Chips oder des 3D-Bioprintings [.] simulieren“. Die Baukosten tragen anteilig der Bund und das Land Berlin, und die Fertigstellung ist für 2023 geplant.[59]

Beleg 3: Verbesserte Entwicklung von Medikamenten

Komplexe tierfreie Modelle wie Organ-Chips werden in der Industrie immer häufiger in der Arzneimittelentwicklung benutzt, beispielsweise zur Untersuchung von arzneimittelinduzierter Leberschädigung.[60]

Eine Publikation des US-amerikanischen Herstellers Emulate aus dem Jahr 2022 zeigte, dass deren humaner Leber-Chip in der Lage ist, arzneimittelinduzierte Leberschäden durch kleine Moleküle vorherzusagen. 27 Medikamente, die laut klinischen Studien mit Menschen entweder schädlich oder harmlos für die Leber sind, wurden in dieser Studie in verblindeter Form verwendet, um die Vorhersagekraft des Organ-Chips zu messen. Bei diesen Medikamenten ging man nach Tierversuchen davon aus, dass sie sicher für die Anwendung am Menschen waren – einschließlich derer, die später beim Menschen toxische Wirkungen auf die Leber zeigten. Der Leber-Chip konnte nahezu 7 von 8 der leberschädigenden Medikamente korrekt erkennen und identifizierte 100 % der nicht leberschädigenden Substanzen richtig als ungefährlich. Wirtschaftlich könnte dies ebenso enorme Auswirkungen haben. Schätzungen dieser Studie zufolge könnte die höhere Produktivität in der Forschung und Entwicklung der Pharmaindustrie jährlich über 3 Milliarden Dollar einbringen.[61]

> „Die Ergebnisse dieser Studie zeigen, dass die Einbeziehung von prädiktiven Organ-Chips in die Prozesse der Arzneimittelentwicklung die Entdeckung und Entwicklung von Arzneimitteln erheblich verbessern könnte, sodass die Hersteller sicherere und wirksamere Arzneimittel in kürzerer Zeit und zu geringeren Kosten auf den Markt bringen könnten.“[61]

Neue Technologien wie diese werden nicht nur die Dauer der Arzneimittelentwicklung verkürzen und den Prozess sicherer, billiger und effektiver gestalten. Sie ermöglichen auch die Bildung interdisziplinärer Forschungsteams, die für die Erstellung personalisierter Krankheitsmodelle für die Präzisionsmedizin oder die Entwicklung effektiver und präziser Systeme für die toxikologische Risikobewertung von grundlegender Bedeutung sind.

V. Regulatorische Möglichkeiten zur Beurteilung der humanen Toxizitätsprüfung

Die Art und Weise, wie chemische Substanzen getestet werden, hat sich in den letzten 25 Jahren grundlegend verändert. Tierversuche werden Schlag auf Schlag mit tierfreien Verfahren ersetzt. Dies beruht auf einem besseren Verständnis der biologischen Prozesse und dem Aufkommen neuer Technologien, die die Entwicklung von Testmethoden ermöglicht haben, welche sich unmittelbar mit zellulären Mechanismen befassen und nicht auf die plumpen und undurchsichtigen Ergebnisse von Tierversuchen angewiesen sind. Aber es ist auch das Ergebnis von öffentlichem Druck und, wie nachstehend erläutert, der Unzufriedenheit von wissenschaftlich Tätigen mit den Ergebnissen aus Tierversuchen. Zelluläre und genetische Informationen über die potenzielle Toxizität einer Chemikalie, wie z. B. das Potenzial für die Rezeptorbindung oder die Aktivierung von Genen oder Signalwegen, lassen sich in tierfreien Versuchen (unter Verwendung menschlicher Zellen, *in vitro*) leichter gewinnen als in Tierversuchen.[62]

Gleichzeitig setzt sich die Erkenntnis bei Aufsichtsbehörden und der regulierten Industrie durch, dass Tierversuche weder die menschliche Gesundheit noch die Umwelt angemessen schützen und dass „der derzeitige Ansatz zeitaufwendig und kostspielig ist und zu einem überlasteten System führt, in dem viele Chemikalien trotz des Potenzials der menschlichen Exposition nicht getestet werden“.[63]
2007 veröffentlichten die US-amerikanischen National Academies of Sciences, Engineering, and Medicine ein wegweisendes Dokument mit dem Titel „Toxicity Testing in the 21st Century: A Vision and a Strategy“.[64] Der Strategie zufolge könnten Fortschritte in den Bereichen Toxikogenomik, Bioinformatik, Systembiologie, Epigenetik und Computertoxikologie die Toxizitätsprüfung grundlegend verändern – von einem System auf der Grundlage von Versuchen am Tier als Ganzes hin zu einem System, das in erster Linie auf *In-vitro*-Methoden beruht, mit denen Änderungen in biologischen Prozessen unter Verwendung von Zellen und Zelllinien oder zellulären Bestandteilen, vorzugsweise menschlichen Ursprungs, bewertet werden. Mit den vorgeschlagenen Änderungen lassen sich bessere Daten über die potenziellen Risiken generieren, denen Menschen durch Umwelteinflüsse wie Pestizide ausgesetzt sind. Das schafft eine stärkere wissenschaftliche Grundlage zur Verbesserung regulatorischer Entscheidungen, um diese Risiken zu senken. Zudem lassen sich Zeit, Geld und die Zahl der in Versuchen eingesetzten Tiere verringern.

Der Bericht empfiehlt einen Ansatz, der das sich rasant entwickelnde wissenschaftliche Verständnis bezüglich der Art und Weise nutzt, wie Gene, Proteine und kleine Moleküle interagieren, um eine normale Zellfunktion zu erhalten, und wie einige dieser Wechselwirkungen auf eine Art und Weise gestört werden können, die zu gesundheitlichen Problemen führen kann. Der neue Versuchsansatz konzentriert sich vor allem auf Toxizitätspfade, sogenannte Adverse Outcome Pathways (AOP). Es handelt sich dabei um zelluläre Prozesse, die voraussichtlich nachteilige Auswirkungen auf die Gesundheit haben, wenn sie entsprechend gestört werden. Das Komitee empfiehlt die Verwendung von Hochdurchsatz-Assays (schnelle, automatisierte Experimente, mit denen Hunderte oder Tausende von Chemikalien in einem breiten Konzentrationsbereich getestet werden können), um die Auswirkungen von Chemikalien auf diese Toxizitätspfade zu bewerten. Auf der Grundlage der Daten aus diesen und anderen Experimenten könnten die Forschenden Modelle zur Beschreibung der Reaktionen auf Toxizitätspfade entwickeln sowie Modelle zur Abschätzung der erforderlichen menschlichen Exposition, um auf diesen Wegen Reaktionen hervorzurufen.[64]

Die derzeitigen Verfahren, mit denen wir neue *In-vitro*-Ansätze validieren, müssen so angepasst werden, dass ihre Fähigkeit, Toxizitätsmechanismen oder spezifische Ereignisse innerhalb eines AOP zu bewerten, miteinbezogen wird. Der traditionelle Ansatz zur Bewertung der Genauigkeit einer neuen Methode erfordert in der Regel einen direkten Vergleich der neuen Daten mit Daten aus Tierversuchen. Dies ist nicht nur wegen der mangelnden Reproduzierbarkeit vieler *In-vivo*-Tests problematisch, sondern auch, weil sie häufig speziesspezifische Ergebnisse liefern, die nicht unbedingt mit der menschlichen Biologie, mit Toxizitätsmechanismen oder spezifischen AOP-Ereignissen korrelieren.[65]

Um mit der rasanten Entwicklung im Bereich der tierfreien toxikologischen Tests Schritt zu halten, ist es darüber hinaus entscheidend, dass Gelder für die Weiterbildung von Behördenmitarbeitenden und Forschenden bereitgestellt werden.
Es ist außerdem von großer Bedeutung, dass Statistiken über die Zahlen der in einzelnen Versuchen verwendeten Tiere geführt werden, um den Bemühungen, Tierversuche zu ersetzen, entsprechend Vorrang einzuräumen und Fortschritte nachverfolgen zu können.

Indem wir den Einsatz von Tierversuchen zu regulatorischen Zwecken, für die ein vollständiger Ersatz vorhanden ist, uneingeschränkt vermeiden und die Akzeptanz der derzeit in der Entwicklung befindlichen Methoden fördern, können wir das Paradigma vorgeschriebener Versuche weiter in Richtung innovativer tierfreier Techniken verlagern und damit in der Anwendung dieser Methoden weltweit eine führende Position einnehmen. In den Anhängen zu diesem Bericht werden Möglichkeiten erörtert, die Verwendung von Tieren in vorgeschriebenen Versuchen sofort oder innerhalb der nächsten zwei bis zehn Jahre einzustellen. Dazu gehören Versuche zu akuten systemischen Erkrankungen, Genotoxizität und Pyrogenität, Impfstoff- und Biologika-Tests, Versuche zu endokrinen Störungen und zu Karzinogenität.

VI. Öffentliche Meinung und die Leidensfähigkeit der Tiere

Die öffentliche Ablehnung der tierexperimentellen Forschung gehört zu den wesentlichen Triebkräften für eine Änderung des Rechtsrahmens. Beispielsweise wurde das Verbot von Tierversuchen für Kosmetika und der Vermarktung von an Tieren getesteten Kosmetikprodukten nach immensem öffentlichem und politischem Druck in ganz Europa in die EU-Kosmetikverordnung aufgenommen – beruhend auf der grundlegenden Überzeugung, dass der Schaden, der den Tieren in Versuchen zugeführt wird, nicht durch den potenziellen Nutzen neuer Kosmetika aufgewogen werden kann.[66]

Mit einer Europäischen Bürgerinitiative zum Thema Tierversuche haben über 1,2 Millionen europäische Staatsangehörige kürzlich die EU-Kommission aufgefordert, das Verbot von Kosmetiktests zu schützen und zu stärken. Zudem fordern sie, die Prüfung von Chemikalien so zu reformieren, dass die Anwendung tierfreier Methoden im Mittelpunkt steht. Außerdem soll sich die EU-Kommission zur Erarbeitung eines konkreten Ausstiegsplans verpflichten, um Tierversuche letztlich zu beenden.[8] Infolgedessen ergreift die Europäische Kommission nun Maßnahmen, um den Übergang zu einer tierfreien Wissenschaft zu beschleunigen. Das beinhaltet auch die Zusicherung, einen Fahrplan für die Abschaffung von Tierversuchen für Industriechemikalien, Pestizide, Biozide sowie Human- und Tierarzneimittel zu entwickeln. In Bezug auf Kosmetiktests hingegen hängen spezifische Maßnahmen noch vom Ausgang eines Verfahrens vor dem Europäischen Gerichtshof ab.[67]

Eine YouGov-Umfrage aus dem Jahr 2009, die in sechs EU-Ländern durchgeführt wurde, ergab eine überwältigende Ablehnung von Tierversuchen: 89 Prozent der befragten Personen aus Deutschland sprachen sich für ein Verbot aller Versuche aus, bei denen Tiere starken Schmerzen und Leiden ausgesetzt sind.[68] Daneben ist auch die öffentliche Unterstützung für Investitionen in tierfreie Testmethoden hoch: In einer Forsa-Umfrage von 2017 unterstützten 69 Prozent der befragten Personen die Forderung, eine Strategie zum Ausstieg aus Tierversuchen in Deutschland zu entwickeln.[69] Außerdem befürworteten 74 Prozent der befragten Personen in einer von der britischen Regierung in Auftrag gegebenen Umfrage verstärkte Anstrengungen zur Entwicklung von Alternativen zu Tierversuchen.[34]

Die Statistik zeigt, dass Tiere in der biomedizinischen Forschung kein geeignetes Abbild für den menschlichen Organismus darstellen. Doch im Hinblick auf ihre Leidensfähigkeit stellt sich die Frage: Wie sehr müssen sie dem Menschen entsprechen, bevor eine kritische Hinterfragung der tierexperimentellen Forschung als zwingend erforderlich erachtet wird?

Angesichts der wachsenden Erkenntnis zur Empfindungsfähigkeit der Tiere ist der öffentliche Widerstand gegen Tierversuche nicht überraschend. Im Jahr 2012 unterzeichnete eine Gruppe anerkannter internationaler Forschender in den Neurowissenschaften die sogenannte „Cambridge Declaration on Consciousness". Darin erklärten die Forschenden ausdrücklich, dass „nicht nur Menschen die neurologischen Grundlagen besitzen, die zur Ausbildung von Bewusstsein führen" und dass, ähnlich wie der Mensch, auch „nichtmenschliche Tiere über die Fähigkeit […] zu intentionalem Verhalten verfügen".[33] Die Erklärung verdeutlicht, dass die Erkenntnis der Empfindungsfähigkeit der Tiere auch in der wissenschaftlichen Gemeinschaft zunimmt. Statistiken zeigen, dass Tiere in der biomedizinischen Forschung kein geeignetes Abbild für den menschlichen Organismus darstellen. Doch im Hinblick auf ihre Leidensfähigkeit stellt sich die Frage: Wie sehr müssen sie dem Menschen entsprechen, bevor eine kritische Hinterfragung der tierexperimentellen Forschung als zwingend erforderlich erachtet wird?

„Der vorsätzliche und routinemäßige Missbrauch unschuldiger, empfindungsfähiger Tiere, der Verletzungen, Schmerzen, Leid, belastende Gefangenhaltung, Manipulation, Handel und Tod umfasst, sollte eigentlich unvorstellbar sein. Tierversuche sind jedoch genau das – die „Normalisierung des Unvorstellbaren."

– Oxford Centre for Animal Ethics

Mehr als 150 wissenschaftlich Tätige sowie gelehrte und schriftstellende Personen unterstützten zudem einen Bericht des Oxford Centre for Animal Ethics, der Tierversuche als ethisch und wissenschaftlich nicht vertretbar verurteilt.[70] Dort heißt es „[d]er vorsätzliche und routinemäßige Missbrauch unschuldiger, empfindungsfähiger Tiere, der Verletzungen, Schmerzen, Leid, belastende Gefangenhaltung, Manipulation, Handel und Tod umfasst, sollte eigentlich unvorstellbar sein. Doch Tierversuche sind genau das – die ‚Normalisierung des Unvorstellbaren'". Sie kommen zu dem Schluss, dass Tierversuche im Widerspruch zu allem stehen, was wir heute über die Fähigkeiten von Tieren wissen. Tiere können nicht nur Schmerzen empfinden, sondern auch unter Schock, Angst, böser Vorahnung, Trauma, Sorge, Stress, Kummer und Schrecken leiden.

VII. Weltweite Führungsposition

Weltweit sind Bewegungen zu verzeichnen, die den wachsenden Konsens in der wissenschaftlichen Gemeinschaft widerspiegeln, dass die Verwendung von Tieren in der biomedizinischen Grundlagenforschung, der Aus- und Weiterbildung oder für die Anforderungen der regulatorischen Bewertung weder ethisch noch wissenschaftlich vertretbar ist. In vielen Teilen der Welt sind grausame und tödlich endende Tierversuche für Kosmetika mittlerweile illegal oder entsprechende Verbote sind in der Entwicklung. Darüber hinaus wurden Tierversuche für Haushaltsprodukte und deren Inhaltsstoffe in Israel und Indien bereits verboten. In Großbritannien hat das britische Innenministerium strenge Beschränkungen bezüglich der Verwendung von Tieren für solche Versuche auferlegt.[71] Die britische Gesundheits- und Sicherheitsbehörde (Health and Safety Executive) hat zudem Tierversuche für Pflanzenschutzmittel erheblich eingeschränkt.[72] In Deutschland untersagt das Tierschutzgesetz grundsätzlich Tierversuche zur Entwicklung von Tabakerzeugnissen, Waschmitteln und Kosmetika.[73]

Die niederländische Regierung kündigte 2016 ihren Plan an, bis 2025 weltweit führend im Bereich der tierversuchsfreien Innovation zu werden. Kurz danach veröffentlichte das niederländische Komitee für den Schutz der für wissenschaftliche Zwecke verwendeten Tiere (NCad) sein Gutachten über den Übergang der Niederlande zu tierfreien Innovationen, in dem es unter anderem zu dem Schluss kam, dass toxikologische Tierversuche für Chemikalien, Lebensmittelinhaltsstoffe, Pestizide, Tierarzneimittel und Impfstoffe bis 2025 auslaufen könnten.[74] Anschließend wurde das Übergangsprogramm für Innovation ohne Tierversuche (TPI) ins Leben gerufen, das Interessengruppen zusammenbringen und eine Plattform für die Entwicklung von

Aktivitäten bieten soll, um den Übergang zu Innovationen ohne Versuchstiere zu beschleunigen.[3]

Die US-amerikanische Umweltschutzbehörde (EPA) hat 2021 ihren Arbeitsplan für neue tierfreie Methoden (NAMs) zur Reduktion von Tierversuchen erstmalig aktualisiert. Der Plan listet konkrete Schritte auf, die die Behörde in den nächsten drei Jahren unternehmen will, um Tests an Wirbeltieren für Pestizide und Chemikalien zu reduzieren. Dazu gehört auch die Vertrauensbildung in NAMs und die verstärkte Einbindung verschiedener Interessengruppen. Der Plan der EPA betont, dass tierfreie Methoden das Potenzial haben, die Gründlichkeit und Ausgereiftheit der chemischen Bewertung durch die Behörde zu erhöhen.[75]

Darüber hinaus wurde im Jahr 2022 mit dem FDA Modernization Act 2.0 das US-amerikanische Gesetz für Lebensmittel, Arzneimittel und Kosmetika dahingehend geändert, dass Tierversuche für neue Arzneimittel nicht mehr zwingend vorgeschrieben sind. Damit wurde festgelegt, dass Verfahren die „am ehesten geeignet sind, die Reaktion des Menschen auf Grundlage der Evidenz wissenschaftlicher Erkenntnisse vorherzusagen“, zellbasierte Methoden, Organchips und mikrophysiologische Systeme, Computermodelle und andere auf der menschlichen Biologie basierende Methoden miteinschließen.[76]

Solche Veränderungen sind erforderlich, damit die Qualität der biomedizinischen Forschung und der regulatorischen Bewertung verbessert wird und Deutschland sich als führendes Land für innovative und überlegene Forschungs- und Versuchsmethoden bewähren kann.

VIII. Maßnahmenplan: Empfehlungen zur Modernisierung der wissenschaftlichen Forschung und Prüfung

1. Die Verwendung von Tieren sollte in jenen Forschungsbereichen unverzüglich eingestellt werden, in denen sich die Ergebnisse aus Tierversuchen nachweislich schlecht auf den Menschen übertragen lassen und in denen Tierversuche den Fortschritt behindern.

Überprüfungen haben wiederholt nachgewiesen, dass Tierversuche in bestimmten Bereichen auf ganzer Linie versagen, wenn es um den Nutzen für die menschliche Gesundheit geht. Zu diesen Bereichen gehören neurodegenerative und neuropsychiatrische Erkrankungen, Herz-Kreislauf-Erkrankungen und Schlaganfälle, Krebs, Diabetes und Adipositas, Entzündungen und Immunreaktionen, die HIV-/AIDS-Forschung, Suchtstudien, die Traumaforschung und die medizinische Ausbildung. Daher sollten Tierversuche in diesen Gebieten schnellstmöglich beendet und durch wirksamere und effizientere tierfreie Methoden ersetzt werden. Im englischsprachigen Anhang werden diese Bereiche eingehender behandelt und entsprechende Empfehlungen ausgesprochen.

2. Mithilfe von kritischen wissenschaftlichen Untersuchungen sollten jene Bereiche ermittelt werden, in denen Tierversuche ebenfalls umgehend eingestellt werden können.

In Untersuchungsbereichen, in denen noch Zweifel daran bestehen, dass der Einsatz von Tieren nicht zielführend ist, sollte eine gründliche systematische Überprüfung durchgeführt werden, um die Wirksamkeit von Tierversuchen zu bestimmen. Systematische

Überprüfungen, in denen verschiedene Forschungsstudien kritisch analysiert werden, sind der erste Schritt zur Beurteilung der Wirksamkeit der tierexperimentellen Forschung und Toxizitätsprüfung. Einige Länder, darunter die Niederlande, schreiben vor, dass systematische Überprüfungen durchgeführt werden müssen, bevor Tierversuche finanziert werden können. Forschende des Universitätsklinikums der niederländischen Radboud-Universität haben vor diesem Mandat die folgende Erklärung veröffentlicht:

> „Wie bei klinischen Studien mit Menschen liegt es in unserer wissenschaftlichen und gesellschaftlichen Verantwortung, auch bei Tierversuchen systematische Überprüfungen routinemäßig durchzuführen. […] Förderorganisationen sollten systematische Überprüfungen anregen und finanzieren. […] Systematische Überprüfungen bringen Unzulänglichkeiten bezüglich der Methodik einzelner Studien ans Licht. Dies trägt dazu bei, das zukünftige Studiendesign zu verbessern und die Fehlerrate bei Tierversuchen mit neuen Arzneimitteln zu senken. Insbesondere können Förderorganisationen im Rahmen einer Finanzierung systematische Überprüfungen von Tierversuchen anordnen. Dies ermöglicht eine stärker evidenzbasierte Auswahl der Tiermodelle und bietet einen besseren Schutz für menschliche Erkrankte.“[77]

Darüber hinaus schreibt Artikel 58 der Richtlinie 2010/63/EU vor, dass die Europäische Kommission regelmäßige Überprüfungen in Bezug auf die Verwendung von Tieren in wissenschaftlichen Verfahren durchführt, wodurch ein klarer Mechanismus zur Förderung des Ersatzes von Tieren in wissenschaftlichen Verfahren bereitgestellt wird. Um mit wissenschaftlichen Innovationen Schritt halten zu können, ist es sehr wichtig, dass dieser Prozess fokussiert und zeitnah abläuft. Um das Potenzial des Prozesses zu maximieren, ist es entscheidend, dass dies in Absprache mit den Mitgliedstaaten und anderen Interessenvertretenden erfolgt.

3. Es sollten transparente, aussagekräftige prospektive und retrospektive Bewertungen gemäß der Richtlinie 2010/63/EU des Europäischen Parlaments und des Rates vom 22. September 2010 zum Schutz der für wissenschaftliche Zwecke verwendeten Tiere durchgeführt werden.

Gemäß Richtlinie 2010/63/EU müssen Anträge auf die Durchführung von Tierversuchen beurteilt werden, um sicherzustellen, dass verfügbare alternative Techniken und Testmethoden uneingeschränkt genutzt werden. Zudem soll geprüft werden, ob das Ausmaß der Schmerzen, Ängste und Leiden, die den Tieren wahrscheinlich zugefügt werden, durch das erwartete Ergebnis gerechtfertigt sind.[7] Auch wenn diese Projektbeurteilungen im Allgemeinen durch staatliche Stellen vorgenommen werden, bieten sie zumindest die Möglichkeit für die Durchführung einer Bewertung nach ethischen Erwägungen. Dennoch kam eine kürzlich durchgeführte rückblickende Analyse von Pandora Pound und Christine J. Nicol zu dem Schluss, dass „die bestehenden Regulierungssysteme die Tiere nicht vor schwerem Leiden bewahren oder sicherstellen konnten, dass nur nützliche, wissenschaftlich strenge Forschung betrieben wurde“.[78] In der Publikation wurden die Leiden, die Tieren in präklinischen Studien für sechs Behandlungsformen zugefügt wurden, mit den Vorteilen, die die Studien für den Menschen boten, verglichen. Sie kamen zu dem Ergebnis, dass weniger als sieben Prozent der Studien hätten genehmigt werden dürfen und dass alle Studien von geringer Qualität waren. Eine Analyse aus Deutschland zeigt, dass 2015-2017 lediglich weniger als 1 Prozent der Tierversuchsvorhaben von den Behörden abgelehnt wurden.[79]

Um die Stabilität des Regulierungssystems zu verbessern, hat das Tierversuchskomitee (Animals in Science Committee) der britischen Regierung empfohlen, die prospektive Schaden-Nutzen-Analyse zu verbessern und gesellschaftliche Bedenken in Bezug auf die tierexperimentelle Forschung zu untersuchen und zu berücksichtigen. Darüber hinaus empfahl der Ausschuss, Methoden zur Vermeidung von Verfahren zu erforschen, die voraussichtlich starke Schmerzen, Leiden und dauerhafte Schäden verursachen – mit dem Ziel, diese Verfahren gänzlich abzuschaffen.

Zusätzlich zu den vorgeschriebenen prospektiven Projektevaluierungen schreibt Artikel 39 der Richtlinie 2010/63/EU auch eine retrospektive Bewertung von Verfahren vor, die als „schwer“ eingestuft sind, sowie von solchen, bei denen nichtmenschliche Primaten verwendet werden (außer Verfahren, deren Schweregrad als „gering“ eingestuft ist oder bei denen die Lebensfunktion nicht wiederhergestellt wird). Dies dient dazu, den Schweregrad rückwirkend beurteilen und feststellen zu können, „ob die Projektziele erreicht wurden“.[7] Die vollständige Umsetzung der seit 2013 geltenden Auflage steht noch aus. Damit die rückblickende Projektbeurteilung jedoch bestimmungsgemäß angewendet werden kann, ist es erforderlich, sie nicht nur als eine bürokratische Pflichtübung zu verstehen. Es bleibt zu hoffen, dass sich der Vergleich der erwarteten Projektziele mit den tatsächlich erzielten Ergebnissen für die künftige Entscheidungsfindung als nützlich erweisen wird. Rückblickende Bewertungen müssen daher öffentlich einsehbar sein und in die nach Artikel 58 der Richtlinie 2010/63/EU erforderlichen thematischen Überprüfungen einfließen.

Um die wissenschaftliche Kontrolle von Forschungsvorhaben zu verbessern und erfolglose „Tiermodelle“ zu ermitteln, empfehlen wir den Mitgliedstaaten, einen soliden Zeitplan für prospektive und retrospektive Bewertungen gemäß den Anforderungen der Richtlinie 2010/63/EU zu erstellen und umzusetzen. Um die Transparenz und Rechenschaftspflicht des Regulierungsprozesses weiter zu erhöhen, sollten Genehmigungsanträge für einen gewissen Zeitraum für öffentliche Stellungnahmen zur Verfügung gestellt werden. Zudem sollten damit verbundene rückwirkende Bewertungen veröffentlicht und mit dem ursprünglichen Antrag in Zusammenhang gebracht werden. Diese Änderungen werden dazu beitragen, die Genauigkeit des Schaden-Nutzen-Analyseprozesses und seine Relevanz für die klinischen Ergebnisse beim Menschen sicherzustellen.

4. Die Harmonisierung und Förderung der internationalen Akzeptanz von tierversuchsfreien Verfahren zur Erfüllung der gesetzlichen Anforderungen an Toxizitätsprüfungen sollte vorangebracht werden.

Wie zuvor beschrieben, ebnet die behördliche Akzeptanz tierfreier Techniken in einer Region oder einem Land den Weg für die internationale Harmonisierung und weitere gesetzliche Abschaffung von Tierversuchen. Aus diesem Grund setzen wir uns dafür ein, dass nationale und internationale Aufsichtsbehörden und Normungsorganisationen mit Industrieunternehmen, Forschungseinrichtungen und einschlägigen Nichtregierungsorganisationen weltweit zusammenarbeiten, um klare Wege zur Validierung und Harmonisierung von tierfreien Techniken für behördliche Prüfanforderungen zu finden und zu fördern und entsprechende Regelwerke zu schaffen.

Wissenschaftliches Vertrauen kann gewonnen werden, indem Fachleute eine transparente Bewertung der Zweckmäßigkeit, technischen Zuverlässigkeit und Relevanz einer neuen Methode durchführen. Die Umsetzung eines klaren Regelwerks für die Bewertung neuer Methoden in der Toxizitätsprüfung, welches diese Schlüsselelemente umfasst, wird eine schnellere Anwendung fundierter wissenschaftlicher Erkenntnisse ermöglichen und fehlerhafte Tierversuche ersetzen. Die Bewertung neuer Methoden sollte vor allem darauf aufbauen, wie gut das Verfahren die menschliche Biologie widerspiegelt, und nicht, wie gut die Ergebnisse mit denen traditioneller Tierversuche übereinstimmen.[65]

Um die Vision eines differenzierten Ansatzes für Toxizitätstests, der Sicherheitsinformationen zu allen im Handel befindlichen Chemikalien angemessener bereitstellt, zu verwirklichen, empfehlen wir Regulierungs- und Regierungsbehörden zudem, die derzeit geltende EU-Rechtsvorschrift und demnach das Tierschutzgesetz, durchzusetzen. Folglich sollte, soweit möglich, anstelle von Tierversuchen eine wissenschaftlich zufriedenstellende Methode oder Versuchsstrategie genutzt werden, die keine Verwendung lebender Tiere beinhaltet.[7,80] Darüber hinaus empfehlen wir, dass die Einrichtung eines öffentlich-privaten Zentrums für prädiktive tierfreie Toxikologie über das EURL ECVAM koordiniert wird. Ein solches Zentrum würde dazu beitragen, die Wissenschaft der Sicherheitsbewertung zu transformieren und neue Instrumente zu entwickeln, mit denen Industrie, Regierung, Konsumierende und internationale Handelsbeteiligte bei der Einführung bewährter Verfahren unterstützt werden.

5. Die finanzielle Förderung von Tierversuchen sollte reduziert und die Mittel für tierfreie Testverfahren sollten aufgestockt werden.

Die schlechte Vorhersagbarkeit von präklinischen Tierversuchen im Hinblick auf die Toxizität und Wirksamkeit beim Menschen hat zu hohen Ausfallraten bei der Entwicklung neuer Therapien geführt und ist wahrscheinlich die Ursache für die unzureichenden Investitionen in die Biowissenschaften. Da sich die EU auf den Übergang von dem Förderprogramm Horizont 2020 zu Horizont Europa konzentriert, sollten die Mitgliedstaaten ihren Schwerpunkt darauf legen, das künftige nationale Wirtschaftswachstum durch die Entwicklung innovativer, intelligenter Technologien und die Förderung externer Investitionen in die Biowissenschaften voranzutreiben. Wie zuvor beschrieben, gehören tierfreie Techniken zu den neu aufkommenden Bereichen mit wachsendem wirtschaftlichem Potenzial. Investitionen in diese Bereiche könnten die Rendite steigern und wiederum Anreize für neue Investoren bieten.

Die nationale Entwicklung dieses Bereichs ist nicht nur finanziell und wissenschaftlich sinnvoll, sondern die EU-Mitgliedstaaten sind gemäß Artikel 47 der Richtlinie 2010/63/EU auch gesetzlich dazu verpflichtet, einen Beitrag zur Entwicklung und Validierung tierfreier Methoden zu leisten, die weitere Forschung auf diesem Gebiet voranzutreiben und Informationen über tierfreie Ansätze zu fördern und zu verbreiten.[7]

Nationale, europäische und internationale Institute müssen nun den nächsten Schritt unternehmen und die Finanzierung widersinniger Versuche beenden, die bislang keine wirksamen Behandlungen und Heilmittel hervorgebracht haben. Mit größeren Investitionen in spannende und innovative tierfreie Methoden und entschlossene politische Initiativen können Heilmittel und Behandlungen für den Menschen entwickelt werden, die weitaus vielversprechender sind. Zudem wird auf diese Weise auch das nahezu unvorstellbare Leid von Millionen von Tieren verringert.

6. Weiterbildung und Schulung von Forschenden und Behördenmitarbeitenden zu den Vorteilen von tierfreien Methoden und deren Anwendung.

Vor dem Hintergrund der zunehmenden Anwendung tierfreier Methoden in der Forschung und Toxizitätsprüfung können verstärkte Aufklärung und eine praktische Ausbildung den Übergang zu diesen Verfahren weiter beschleunigen. Bei der Umsetzung solcher Initiativen sollte beachtet werden, dass bei der Einführung neuer Technologien Hindernisse auftreten können und daher Anstrengungen zur Vertrauensbildung in diese erforderlich sind. Um ein wesentliches Hindernis für die Anwendung tierfreier Methoden aus dem Weg zu räumen, ist es laut der britischen Innovationsagentur „Innovate UK" erforderlich, zunächst die Skepsis gegenüber tierfreien Methoden und ihrer Fähigkeit zur Modellierung biologischer Prozesse zu überwinden. Starre Strukturen und konservative Denkweisen, die die Abkehr von tiergestützten Methoden behindern, lassen sich überwinden, wenn Forschende darin bestärkt werden, „sich Gedanken zu Aspekten zu machen, die über ihren unmittelbaren Forschungsbereich hinausgehen, und zu überlegen, wie sie ihre Fähigkeiten, Technologien und Expertise nutzen können, um die Entwicklung und Einführung fortschrittlicher tierfreier Methoden zu beschleunigen".[81] Solche Bildungsinitiativen sollten von angehenden Forschenden bis hin zu etablierten Fachleuten in der gesamten Branche angenommen und mit ausreichender finanzieller Unterstützung versehen werden. Dazu zählen die akademische Forschung, die Industrie, die Behörden sowie Wissenschafts- und Förderorganisationen.

Es besteht Bedarf an zusätzlicher Weiterbildung und praktischer Ausbildung im Bereich der tierfreien Methoden. Damit die EU mit den internationalen Entwicklungen Schritt halten kann, müssen Studierende und Nachwuchsforschende außerdem verstärkt die Möglichkeit erhalten, die notwendigen Fähigkeiten zu entwickeln, um einen Beitrag zu diesem Forschungsbereich zu leisten. Da in vielen Studiengängen nicht genügend Kurse zu tierfreien Methoden angeboten werden, wurden ergänzende Ausbildungsprogramme entwickelt. So veranstaltet beispielsweise die Gemeinsame Forschungsstelle der EU-Kommission (Joint Research Centre, JRC) regelmäßig eine Summer School zu tierversuchsfreien Methoden.[82] Ähnliche Programme könnten auch auf nationaler Ebene aufgelegt werden. In Kanada etwa hat die University of British Columbia ein neues, von der Society for Humane Science angebotenes Modul über tierfreie Methoden in der biomedizinischen Forschung aufgenommen, das sich auf die Ausbildung von Studierenden in tierfreien Forschungs- und Testmethoden konzentriert.[83] Darüber hinaus gibt es viele Online-Angebote von Fachleuten auf diesem Gebiet, u. a. vom PETA Science Consortium International e.V. und dem Physicians Committee for Responsible Medicine.[84,85] Informationen über tierfreie Forschung und Testung sind demnach verfügbar und sollten Bestandteil der gesamten biomedizinischen Ausbildung sein.

Das Bewusstsein für tierfreie Methoden innerhalb der wissenschaftlichen Gemeinschaft könnte auch durch weitere Maßnahmen gestärkt werden, darunter etwa die Gründung eines nationalen Kompetenzzentrums für tierfreie Forschung und Toxizitätsprüfung oder die Einrichtung von Lehrstühlen und Professuren zu tierfreien Methoden. Daneben könnte es auch hilfreich sein, Beauftragte für tierfreie Forschung zu benennen, die Professorinnen, Mitarbeitende und Studierende beraten können. Universitäten und andere akademische Einrichtungen könnten auch darin bestärkt werden, abteilungsspezifische Gremien für den Übergang zu tierfreier Forschung zu bilden, die bereichsübergreifend arbeiten und beraten. Diese Gremien könnten zur Organisation von Doktoranden- und anderen postgradualen Studiengängen, die ausschließlich tierfreie Methoden anwenden, beitragen sowie Workshops, Seminare und Sommerkurse zu *In-vitro*- und *In-silico*-Methoden anbieten.

Da sich die tierversuchsfreie Wissenschaft und Technologie rasch weiterentwickeln, sind Aus- und Weiterbildungsmaßnahmen nicht nur an Universitäten erforderlich. Der Lehrplan für anerkannte Qualifikationen wie der „European Registered Toxicologist" sollte auch Pflichtkurse über neue Methoden, *In-vitro*-zu-*in-vivo*-Extrapolation, systematische Überprüfungen und AOPs umfassen. Darüber hinaus sollten etablierte Forschende und Behörden, die tiergestützte Methoden anwenden bzw. überwachen, Weiterbildungsmöglichkeiten erhalten. Außerdem sollten sie darin bestärkt werden, multidisziplinäre Kooperationen einzugehen, damit sie ihre Fähigkeiten weiterentwickeln und neue, innovative Wege finden können, um Forschungsfragen und Methoden zur Beantwortung dieser Fragen anzugehen. So hat beispielsweise die niederländische TPI eine Reihe von „Helpathons" ins Leben gerufen.[86] Dabei handelt es sich um interaktive Workshops, die sich um eine bestimmte Forschungsfrage drehen und die Forschenden durch ein Gemeinschaftsforum darin unterstützen, kreativ zu denken und auch zufällige Entdeckungen als neue Möglichkeiten für tierfreie Ansätze zu nutzen.

Förderorganisationen könnten regelmäßige Schulungen von Antragsstellenden verlangen, in denen die vielversprechendsten tierfreien Methoden mit kommerziellem Potenzial vermittelt werden. Ebenso sollten die für die Genehmigung von Tierversuchen zuständigen Fachleute von Aufsichtsbehörden im Rahmen ihrer kontinuierlichen beruflichen Weiterbildung an verpflichtenden Schulungen zu Fortschritten in der tierfreien Wissenschaft teilnehmen. Dies sollte auch jene Personen betreffen, die Testdaten zur Erfüllung gesetzlicher Vorschriften verlangen, z. B. für Human- und Tierarzneimittel, Chemikalien, Biozide und Pestizide.

Da sich der Bereich der tierversuchsfreien Methoden zunehmend ausweitet, müssen Forschende und Regulierungsbehörden mit diesen entscheidenden Entwicklungen Schritt halten. Verstärkte Ausbildungs- und Weiterbildungsmaßnahmen sind daher dringend erforderlich, um Vertrauen in zuverlässige und relevante tierfreie Methoden aufzubauen, die die menschliche Gesundheit und die Umwelt am besten schützen können.

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Appendices

Further details on opportunities to replace animals in the areas of biomedical research and training, forensic sciences, toxicity assessment, and laboratory production methods are described below. Also included is information regarding the expertise of PETA scientists. The appendices feature several examples of the implementation of non-animal methods. However, they do not represent a complete collection of the scientific literature or regulations worldwide.

Any mention of PETA Science Consortium International e.V. prior to December 2020 refers to PETA International Science Consortium Ltd.

Table of Contents

Glossary

3Rs	Replacement, reduction, and refinement
AD	Alzheimer's disease
AIDS	Acquired immunodeficiency syndrome
ALS	Amyotrophic lateral sclerosis
AOP	Adverse outcome pathway
AWA	Animal Welfare Act
BCOP	Bovine corneal opacity and permeability
CAR	Chimeric antigen receptor
CAGR	Compound annual growth rate
CDC	Centers for Disease Control and Prevention
CTA	Cell transformation assay
CVD	Cardiovascular disease
ECHA	European Chemicals Agency
EDQM	European Directorate for the Quality of Medicines & HealthCare
EMA	European Medicines Agency
EPA	US Environmental Protection Agency
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
EViR	Ensuring Value in Research funders' forum
FBS	Foetal bovine serum
FDA	US Food and Drug Administration
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GI	Gastrointestinal
HD	Huntington's disease
hiPSCs	Human induced pluripotent stem cells
HIV	Human immunodeficiency virus
HREA	Health Research Extension Act of 1985
IACUC	Institutional Animal Care and Use Committee
IATA	Integrated approach to testing and assessment
IBD	Irritable bowel disease
IBS	Irritable bowel syndrome
ICAPO	International Council on Animal Protection in OECD Programmes
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICCR	International Cooperation on Cosmetics Regulation
ICCS	International Collaboration on Cosmetics Safety
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ISO	International Organization for Standardization
JRC	European Commission Joint Research Centre
LAL	Limulus amebocyte lysate
MAT	Monocyte activation test
NAGMSC	National Advisory General Medical Sciences Council
NGRA	Next Generation Risk Assessment
NICEATM	US NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
NIGMS	National Institute of General Medical Sciences
NHP	Nonhuman primate
NRU	Neutral red uptake
NTP	US National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OIG	Office of the Inspector General
OPP	Office of Pesticides Programs
OPTN	Organ Procurement and Transplantation Network
PARC	Partnership for the Assessment of Risks from Chemicals

PD	Parkinson's disease
PETA	People for the Ethical Treatment of Animals
Ph Eur	European Pharmacopoeia
QSAR	Quantitative structure-activity relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RhCE	Reconstructed human cornea-like epithelium
RHE	Reconstructed human epidermis
RPT	Rabbit pyrogen test
R&D	Research and development
SA	Structural alert
SCCS	Scientific Committee on Consumer Safety
SCI	Spinal cord injury
SIV	Simian immunodeficiency virus
SUD	Substance use disorder
TBI	Traumatic brain injury
UNOS	United Network for Organ Sharing
USDA	United States Department of Agriculture
WoE	Weight of evidence

Basic and Applied Biomedical Research

Please find in the following pages further details on opportunities to end the use of animals in specific areas of biomedical research. They feature several examples of the implementation of non-animal methods. However, they do not represent an exhaustive account of the scientific literature or developments worldwide.

Cancer

Although improvements in screening programs have significantly advanced early cancer detection and reduced mortality rates,[1,2] cancer remains the second leading cause of death in the U.S., with officials estimating over 600,000 Americans deaths from cancer in 2024.[3] Decreased incidence of cancers over the past two decades has been partially attributed to specific lifestyle changes, such as reduced smoking, increased physical activity, and maintenance of stable body weight.[4,5] Though biomedical research has made some strides in understanding carcinogenesis, clinical trials have failed to translate from the laboratory to the clinic effectively. Even after significant investment in research for cancer therapies, the success rate for oncology drugs is lower than 10%.[6]

A recent meta-analysis showed that cancer experiments on animals have smaller effect sizes and are less likely to replicate than non-animal cancer experiments.[7] Oncologists have noted that "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."[8] Former director of the National Cancer Institute, Dr Richard Klausner, stated, "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."[9] In addition, the enormous pain and suffering experienced by animals raises ethical and welfare concerns.[10,11]

There are several methods by which rodents—predominantly mice—are used in cancer experimentation. These methods are categorized based on the tumour development mechanism: xenografting, genetic engineering, or, less frequently, spontaneous induction through exposure to carcinogenic agents.[12,13]

To create xenografted animals, immortalized or patient-derived human cancer cells are transplanted either under the skin or into an organ of immunocompromised rodents, who may then be subjected to a range of experiments, such as treatment with a drug candidate or a substance of interest. Although xenografting is the most common approach to generate tumours in rodents, an analysis of 1,110 mouse xenograft tumour models concluded that these models face fundamental challenges that hinder their ability to predict therapy outcomes in humans.[14] Transplantation of human cells alters the genetic landscape of mice in ways that are unlikely to happen in humans, and these changes alter responses to drug treatment.

Genetically modified (transgenic) mice are created by inserting or deleting human genes into a mouse's DNA to induce the expression of oncogenes or inactivate tumour-suppressing genes, respectively. Since these modifications happen randomly, researchers cannot control gene expression, and off-target alterations are common.[15] Transgenic mouse cancer models fail to mimic the sporadic nature of tumour development, resulting in unexpected outcomes that would not be present in human patients. Moreover, these models are time-consuming and costly since they require many animals to obtain the desired and stable genotype, and the "surplus animals" are euthanized.[16]

In August 2021, the European Commission's Joint Research Centre published a report on immuno-oncology. It highlighted promising human-based, non-animal methods for developing new therapies, studying cancer biology and immunomodulation, identifying specific molecular biomarkers, and more.[17] Some examples of these human-relevant models for cancer research include three-dimensional platforms, such as bioprinted tumours using patient samples,[18-21] organs–on-a-chip models for precision medicine using different cancer cell lines,[22-26] and patient-derived organoids.[27-29] In addition, cancer genomic datasets[30-34] and machine learning tools[35-38] are available to improve diagnosis and predict responses to therapies in real-time.

Scientists using non-animal methods for cancer research face a smaller translational hurdle since they can use patients' own cancer cells and because these human-relevant methods are grounded in human, not rodent, biology.[39] These new tools and approaches will advance cancer research, produce human-relevant results, and accelerate the field toward precision medicine, but only if funding for them is increased and allocated away from cancer experiments on animals.

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Cardiovascular Disease

Cardiovascular diseases (CVD) are the number one cause of death in the U.S. and worldwide, claiming approximately 17.9 million individuals every year, with mortality rates expected to continue to rise.[40] Despite the availability of therapies for treating CVD, the failure rate of new drugs for CVD treatment was about 75% as of 2022, primarily due to the limitations of animal models in drug discovery and testing.[41] A review of 121 studies using animals for human CVD research found that 79% failed to be replicated in human trials.[42]

Experimenters use a variety of animal species, from frogs to rats to cows, in an effort to study human CVD. Yet, the aetiology and pathology of CVD in these animals often differ significantly from that of humans.[43,44] Most species have distinct cardiovascular functional and structural parameters, including resting heart rate, action potentials, protein isoforms, contraction, and force-frequency response.[45-47] They also exhibit species-specific genetic mechanisms that affect their susceptibility to CVD and responses to drugs intended for human treatment.[48-50] For example, rodents are resistant to atherosclerosis,[51] a key component of CVD. Coronary artery disease, which leads to atherosclerosis, rarely occurs in animals and is difficult to induce, often requiring surgical or pharmaceutical interventions that are not relevant to the human context.[52]

Additionally, behavioural and environmental risk factors, such as diet, physical inactivity, smoking, and air pollution,[53] are complex and not reliably reproducible in animals. These factors contribute to the limited relevance and poor clinical translation of CVD experiments on animals. As recent study's authors noted that "profound understanding of disease progression is limited. The lack of biologically relevant and robust preclinical disease models that truly grasp the molecular underpinnings of cardiac disease and its pathophysiology attributes to this stagnation."[54]

Human-relevant in vitro and in silico methods are more suitable for cardiovascular research, as they reflect human biology better than animal models. Researchers have generated heart organoids using human induced pluripotent stem cells (hiPSCs) that mimic the cellular composition of the heart and self-organize to create chamber-like structures. These heart organoids can recapitulate functional impairments seen in conditions such as cardiac fibrosis and hypertrophic cardiomyopathy.[55-57] A team of engineers in Taiwan has developed a microfluidic chip system to rapidly quantify four CVD biomarkers aimed at improving early intervention.[58] A recent study demonstrated that heart-on-a-chip technology can be used to model cardiac arrhythmias.[59,60] Additionally, machine learning techniques, in combination with patient data, can create models to predict CVD risk, enabling earlier identification of diseases and more effective treatment outcomes.[61-63] Scientists and clinicians have collaborated to develop an algorithm that predicts 10-year disease progression in hypertrophic cardiomyopathy using clinical data.[64] Finally, in silico modelling and simulation can be employed to assess the mechanistic understanding of cardiac pathophysiology.[65] These methods are valuable platforms for studying the human heart, identifying and screening drugs for CVD treatment, and application in regenerative and personalized medicine.

Considering that "[t]here is no ideal animal model available for cardiac research,"[66] CVD research must evolve toward modern methods that rely on human cells and patient-derived data. These new experimental models are more cost-effective and better recapitulate human physiology.[67] Non-animal research methods provide more accurate biological insights into cardiac function, enhancing the translation of preclinical findings into human benefits compared to animal models.[68-70]

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Cell Therapy

Adoptive cellular therapy (cell therapy) involves transplanting human cells to repair or replace damaged tissue. It uses various cell types, such as hematopoietic stem cells, mesenchymal stem cells, and immune cells, harvested from patients themselves (autologous) or donors (allogeneic), to treat a range of conditions.[71,72] Cell therapy has been explored for treating blood-related diseases, solid cancers, and diabetes, as well as for applications in regenerative medicine.[73-77]

Cell therapy research is often conducted using animals, primarily genetically engineered mice, and faces significant limitations. Experiments on animals typically use young, healthy animals who do not reflect the complex aetiology of human diseases that are often influenced by age and other co-morbidities. Additionally, experiments on animals lack the long-term analysis and follow-up needed to assess efficacy in humans, posing a challenge in predicting outcomes.[78] Additionally, immune and physiological differences between species lead to poor translation of results.

Though some cell therapies have been approved for use, these treatments still face challenges, especially for solid cancers, due to tumour heterogeneity and the scarcity of tumour-specific antigens.[79] Engineered chimeric antigen receptor (CAR) T-cell therapies have shown antitumor activity in experiments on mice but failed to work in human clinical trials for ovarian and metastatic renal cell cancers.[80,81] One cause for these failures is that preclinical studies are often conducted using immunocompromised mice with xenografted human tumours, whereas, in clinical practice, these cells operate within a patient's complex and intact immune system.[82] For more on the problems with xenograft mouse models, see the section on Cancer (p.23).

Because animals do not accurately replicate human biology, they may also fail to reliably predict adverse effects of cell therapies, such as cytokine release syndrome and immune effector cell-associated neurotoxicity. Additionally, variability in cell preparation and characterization during preclinical experiments on animals can result in inconsistent and irreproducible findings.[83]

Non-animal preclinical methods for studying and testing cell therapies include in vitro models, such as organoids and those using hiPSCs. These models replicate human physiology more accurately, allowing for

high-throughput drug screening, identification of human-specific mechanisms, and personalized medicine approaches.[84,85] Maulana et al. introduced a patient-derived breast cancer-on-chip model that enables real-time monitoring of CAR T-cell activity and prevention of cytokine release syndrome with an FDA-approved drug.[86] In another study, researchers using patient samples and clinical data identified CD22 as a potential marker for CAR T-cell therapy development in triple-negative breast cancer, which, despite ongoing cell therapy clinical trials, is currently without targeted therapy.[87,88]

Interest in adoptive cell therapies has surged in the past decade and continues to expand to various cancers and diseases. Recent advances in engineering technologies, human in vitro models, and combination therapies are enhancing cell therapy development, providing robust platforms for studying disease mechanisms and therapeutic interventions, and yielding more applicable results.

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Diabetes

For many years, experimenters have intentionally created symptoms of diabetes mellitus (diabetes) in rodents, pigs, dogs, and primates.[89] However, these models face considerable limitations, such as differing disease progression compared to humans. Experimenters attempt to replicate diabetes pathology in animals by inducing symptoms through poor diet and chemical or viral destruction of pancreatic beta cells, but these efforts consistently fail due to significant limitations, such as tissue necrosis and species-specific differences in susceptibility to diabetes.[90,91]

Beyond technical limitations, using animals to study diabetes also poses significant biological limitations regarding anatomy, physiology, and exposure.[92,93] For instance, mice rely principally on the liver for glucose homeostasis, while, for humans, skeletal muscle is also critical in glucose metabolism.[94] In addition, some transgenic mice models of type 2 diabetes are based on leptin deficiency, which is not an essential contributor to diabetes in humans.[95] Because of a low rate of spontaneous diabetes (only 2%), the LEW-iddm rat model for type 1 diabetes requires compensatory alterations in the rat's immune cell repertoire in order to develop a diabetic profile but still does not entirely mimic the human condition.[96,97] In the same way, the human pancreas differs from that of rodents in its tissue architecture, cellular composition, and insulin regulation.[98]

Many drugs developed to treat diabetes have adverse side effects, such as oedema, cardiac risk, and weight gain, with some drugs being withdrawn from the market.[99,100] Recent findings reveal significant human singularities in pathology, environment, ethnicity, and treatment responses among type 2 diabetes patients,[101-104] highlighting why the heterogeneity of diabetes cannot be replicated using animals. As a result, experiments on animals have not led to transferable findings for humans.[105,106]

As interspecies differences continue to emerge, there is a clear need for human-based methodologies to advance diabetes research to bridge the gap between pre-clinical and clinical trials and discover new ways to prevent disease progression.[107-109]

Numerous organ-on-a-chip models for studying insulin resistance and glomerular function for diabetic nephropathy have been developed to uncover biological mechanisms and provide insights into effective therapeutic opportunities. For example, a glomerulus-on-a-chip using human cells allows researchers to assess high glucose-induced kidney damage.[110] In another study, the glomerulus-on-a-chip mimicked the human in vivo kidney response to injury in patients exposed to serum and toxic agents, providing a valuable tool to investigate renal damage.[111] Another 3D model used cadaveric pancreas islets for continuous insulin measurements, offering a scalable model to study diabetes and perform drug screening.[112] In silico modelling using diabetic patient data is also showing promising results.[113-115] For example, a model designed to quantify endogenous and inhaled plasma insulin after a meal was tested in a clinical study with healthy patients and can help estimate the bioavailability and pharmacokinetics of inhaled insulin in humans.[116]

Many other human 3D models are being explored for drug development and considered for future organ transplantation in diabetic patients,[117,118] including stem cells[119,120] and pancreatic islets.[121-123] These innovative approaches, based on patient-derived cells, have the potential to accelerate research on diabetes as they permit investigation into the underlying biological mechanisms of human diabetes-induced complications, which are impossible to replicate in experiments on animals.[124,125]

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Inflammation and Immunology

The use of animals in research to study human inflammation and immunology encompasses a great deal of basic and disease-related research. We will briefly discuss three main areas: the use of animals for HIV/AIDS research, the use of mice for human immune research, and the use of animals to study human sepsis.

HIV/AIDS

The failure to translate experiments on animals into effective human applications of human immunodeficiency virus (HIV) vaccines was acknowledged more than 20 years ago when, in 1995, NIH instituted a moratorium on breeding chimpanzees, the species most commonly used in HIV and acquired immunodeficiency syndrome (AIDS) research at the time, recognizing that studies using this species had failed to produce clinically useful data. Following this, experimenters began to use other nonhuman primate species, notably macaques.

Because humans are the only primates who contract HIV and develop AIDS, experimenters instead infect monkeys with simian immunodeficiency virus (SIV), a virus unique to African primates. The genetic homology between HIV and SIV is only 55%, and SIV is less genetically diverse than HIV.[126,127] Owing to differences in surface proteins and other molecular markers, antibodies that neutralize SIV have no effect on HIV, and vice versa.[128] Importantly, the dose of SIV administered to a nonhuman primate in an experiment is often much

higher than the typical amount of HIV-1 to which a human is exposed during sexual transmission.[129] Sometimes, experimenters use an engineered SIV/HIV concoction. AIDS researcher Mark Girard has stressed, "One should realize that we still do not know how the SIV or SHIV model compares to HIV infection in humans. Extrapolating from vaccine protection results in nonhuman primate studies to efficacy in man may be misleading."[130]

Even those who use nonhuman primates as models of HIV have admitted that they "do not allow direct testing of HIV vaccines" and that "because of the complexity and limitations of the NHP [nonhuman primate] models, it remains difficult to extrapolate data from these models to inform the development of HIV vaccines."[131] Experimenters have developed dozens of vaccines candidates using monkeys, but all have failed in human trials.[132] At least two clinical trials resulted in an increased likelihood of HIV infection in humans.[133,134] After one of the failed vaccine trials, Anthony Fauci, former director of the U.S. National Institute of Allergy and Infectious Diseases, acknowledged that the original positive results of a macaque study "might be a fluke."[135]

Scientists have noted that "Existing animal models predicting clinical translations are simplistic, highly reductionist and, therefore, not fit for purpose."[136] They reported 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 concerning congruency with and extrapolation of findings for human hosts."[137]

Because of broad failures in nonhuman primate HIV/AIDS research, some experimenters have shifted their focus to mice—a species even more genetically removed from humans. The "humanized" mouse model for HIV/AIDS research is a mouse who has been partially repopulated with human immune cells, allowing for the animal to be infected with HIV-1. However, humanized mice are limited in their longevity with the disease and retain parts of their murine immune systems, "complicating immune response interpretations."[138] Not surprisingly, the use of humanized mice has also failed to generate valuable results for clinical HIV/AIDS treatment.

Considering the differences between a laboratory environment and human society, experiments on animals will never capture the complexity of this human disease. Mice and rats used in experiments are kept in conditions where the primary pathogens are those found in their faeces, and cofactors that may be present in human patients, such as other microbial infections, are absent. This lack of cofactors significantly alters the acquisition and progression of the virus.[139] Nonhuman primates used in HIV research, on the other hand, have been found to harbour confounding infections like valley fever, which compromises the findings of HIV studies.[140]

Scientists acknowledge that even after costly and unreliable experiments on animals, human data are still needed to determine whether a drug is fit for the clinical setting. Researchers with the U.S. Military HIV Research Program noted 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"[141] adding to this 2007 comment from the associate editor of The BMJ: "When it comes to testing HIV vaccines, only humans will do."[142] Researchers recognize that human in vitro models are needed to replicate this human disease and develop treatments.[143]

Recent non-animal HIV research includes interactive molecular dynamics simulations to predict how drug molecules will bind to HIV proteins,[144-147] novel imaging techniques revealing previously unknown aspects of HIV structure that open up the potential for new therapies,[148] and bioinformatics analysis of specimens from individuals with viremia and in vitro–infected cells from healthy donors to construct an atlas of HIV-susceptible cell phenotypes.[149] Additionally, single-cell multi-omics analyses of samples from healthy and HIV-infected donors have uncovered differences in T cell populations, protein expression, and glycan expression, which could be instrumental in developing novel immune-targeted therapeutic strategies.[150-152]

Scientists around the world have been studying the immune cells of individuals called "HIV controllers," who can become infected with HIV but can control the spread of the virus without any therapeutic intervention.[153-157] 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.[158]

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Mouse Immunology

Since the advent of inbred mouse strains in the 1940s and the development of transgenics in the 1980s, mice have been used in alarming numbers for immunology research. Beyond the ethical concerns these numbers raise, most findings generated by these experiments fail to translate to humans and are not replicable.[159,160]

Key physiological and cellular differences between the tissues of mice and humans reveal their inadequacy as human experimental stand-ins and should disqualify the use of mice in experiments.[161,162] Specifically for immunological research, mice have unique dendritic epidermal T cells with sensory functions non-existent in humans.[163] Similarly, the composition of immune cells in human blood (55-70% neutrophils, 20-40% lymphocytes)[164] is different than that of mice used in experiments (20-30% neutrophils, 70-80% lymphocytes),[165] which affects species-specific immune defence mechanisms.[166,167] Logically, these differences make sense, given that we humans have longer life spans8 and we “do not live with our heads a half-inch off the ground.”[168]

Mice have a unique genetic makeup that contributes to their phenotypic dissimilarities with humans, such as the lack of class II human leukocyte antigen expression on T lymphocytes and differences in the activation of these cells during immune response.[169] These immunological specificities, along with epigenetic modifications unique to mice, hinder the data translation and make comparisons between mice and humans unrealistic and risky.[170,171] For example, a deficiency of CD28 molecules results in severe immune dysfunction in mice, while humans with this deficiency remain healthy.[172] Due, in part, to differences in CD28 expression between species,

clinical trials with Fialuridine resulted in organ failure in humans taking only 1/500th of the dose that had been deemed safe in preclinical tests using animals.[173]

A mouse's immunological layout is also altered by the barren, controlled housing conditions in which they are kept in laboratories. Consequently, mice develop a gut microbiome adapted to these conditions,[174] which is distinct from that of wild mice and even more divergent from humans.[175] In a study that analysed over 1900 mouse genomes, researchers revealed that humans and mice have only 2% of gut bacteria species in common.[176] The breeding process used to generate specific mouse strains with genetic variations also makes them more susceptible to human pathogens than humans, adding another point of discrepancy.[177,178] Mice in laboratories fail to represent the genetic variability found among humans or their own species' wild counterparts.[179,180] Despite these many glaring disadvantages, mice continue to be used for immunological research.

Human immunological research is slowly but surely bringing the "human" back into its focus. "Big data" and computational biology – proteomics, metabolomics, and clinical data – integrated with novel 3D models can bridge the gap in translational science and leverage personalized approaches.[181-184] Human samples, such as bone marrow,[185] lymph nodes,[186] tonsils,[187] and liver,[188] are being used to generate patient-derived organoids to address mechanistic and hypothesis-driven immunological studies in different contexts.

A review summarizing the progress of immune-competent human skin disease models recognizes that the failures of experiments on animals to translate into effective treatments for diseases such as fibrosis, psoriasis, cancer, contact allergy, and autoimmune diseases, is due, in part, to the immunological nature of these conditions. The authors go on to describe how co-culture, three-dimensional organotype systems, and organ-on-a-chip technology will "enable human models of well-controlled complexity, yielding detailed, reliable data, providing a fitting solution for the drug development process."[189]

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Sepsis

Sepsis is a life-threatening condition caused by the body's response to infection. The most recent global incidence data show that sepsis affected an estimated 48.9 million humans worldwide and resulted in 11 million deaths in 2017.[190] It is a leading cause of death in U.S. hospitals and one of the most expensive conditions to treat.[191,192]

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.[193] The difficulty in reliably translating results from mice to humans is considered a primary cause of the failure of nearly all human trials of sepsis therapies.

In 2013, Proceedings of the National Academy of Sciences of the United States of America published a landmark study that took 10 years to complete 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 from hundreds of human clinical patients with results from experiments on animals to demonstrate that humans and mice are dissimilar in their genetic responses for severe inflammatory conditions such as sepsis, burns, and trauma.[194]

Former 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."[195] The 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!*[196]

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 primarily germ-free settings. In contrast, it is mostly infant and elderly humans who live in a variety of unsterilized, unpredictable environments who develop sepsis.[197,198] When experimenters induce the condition in mice, the onset of symptoms occurs within hours to days, whereas in humans it takes day to weeks. Mice are not typically provided with the supportive therapy that human patients receive, such as fluids, vasopressors, and ventilators.[199] Unlike humans, mice are rarely given pain relief,[200] 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, a procedure in which experimenters cut open a mouse's abdomen and puncture their intestines with a needle before sewing the animal back up. However, mice's responses to this procedure vary depending on age, sex, strain, laboratory, the size of the needle used, and the size of the incision, which makes results incomparable between laboratories.[201,202] In addition, the procedure causes the formation of an abscess, whose effects may disguise or be disguised by the effects of the sepsis itself.[203] This means that an intervention that appears beneficial for sepsis may only appear beneficial because of its effects on the abscess.

Rats, dogs, cats, pigs, sheep, rabbits, horses, and nonhuman primates, including baboons and macaques, have also been used in sepsis experimentation. None of these species reproduce 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.[204] Furthermore, baboons and mice are less sensitive to a species of bacteria commonly used to induce sepsis in experimental settings.[205] A recent study found that rhesus macaques and baboons differ markedly in their innate immune response to pathogens compared to humans.[206]

A 2019 report from the National Advisory General Medical Sciences Council (NAGMSC) Working Group on Sepsis states, "Despite decades of intensive study of the underlying mechanisms of this condition, no new drug or significantly new diagnostic technology has emerged. Dozens of prospective trials of agents or strategies

targeting the inflammatory basis of sepsis have failed."[207] In its report, the NAGMSC Working Group on Sepsis recommended that the National Institute of General Medical Sciences (NIGMS), under NIH, "rebalance" its sepsis research–funding portfolio to "include a more clinical focus."[208] In a "Notice of Information" issued by NIGMS following the NAGMSC report, the institute expressed its intention to support sepsis research that "uses new and emerging approaches, such as clinical informatics, computational analyses, and predictive modelling in patients, and new applications of high-resolution and high-throughput bioanalytical techniques to materials obtained from septic patients" and called the support of "[s]tudies using rodent models of sepsis" a "low priority."[209] More recently, at the 2024 Shock Society Annual Conference, NIGMS announced that they were "unwilling" to fund projects proposing mouse models of human sepsis and encouraged the use of animal-free research methods moving forward.[210] In other words, NIGMS intends to prioritize funding human-relevant sepsis research over sepsis experiments on animals. However, other NIH institutes and funders have yet to follow NIGMS' lead.

In 2015, an expert working group consisting of veterinarians, animal technologists, and scientists issued a report on implementing the 3Rs (the replacement, reduction, and refinement of animal use) in sepsis research.[211] The group identified several methods that could be used instead of animal models, including in vitro cell culture models for studying sepsis mechanisms, systems and computation biology for revealing the inflammatory processes occurring during sepsis, three-dimensional cell culture models to explore human disease progression and infectious mechanisms, synthetic human models to recreate disease-related cell types and tissues, and human genomic data to understand 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."[212]

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

- Scientists in Tokyo, Japan, used hiPSC-derived liver organoids to model the pathological events of septic-associated liver dysfunction and recovery following infection.[213]
- A team of engineers, doctors, and researchers at Temple University identified an association between neutrophil types and the severity of sepsis using a human lung-on-chip model, which can be used to determine the appropriate therapeutic intervention based on sepsis severity.[214]
- Researchers in Hefei, China collaborated with physicians at First Affiliated Hospital to create a six-unit microfluidic device that comprehensively analyses of a sepsis patient's white blood cell activity to monitor disease progression and severity.[215]
- Massachusetts General Hospital scientists and physicians created a microfluidic device to accurately detect a biomarker of sepsis pathophysiology using a drop of blood, aiming to improve disease monitoring.[216]
- Because early detection of sepsis is likely the most critical factor in reducing mortality from this condition,[217] researchers around the globe are exploring different artificial intelligence and machine learning tools to aid in sepsis early prediction and diagnosis.[218-226]

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Gastrointestinal Disorders

Gastrointestinal (GI) disorders affect more than a million individuals in the United States and account for millions of clinical visits annually, with health expenditures totalling $119.6 billion in 2018.[227] The burden of these diseases is staggering as they contribute significantly to morbidity, mortality, and healthcare costs, with the prevalence expected to rise.[228] Because of this, tremendous effort has been put into GI disorder drug development, but for many conditions, there has been little success.[229] Treatments are available for GI diseases, but they often entail significant drawbacks, partly because much of the mechanistic knowledge of these diseases has relied on animal models.

Key differences in nonhuman animals render them inappropriate models for studying human GI diseases. The two species most often used in these experiments are rats and pigs.[230] Both have GI tracts that are anatomically dissimilar to humans. For example, the jejunum constitutes 90% of the rat's small intestine but only 38% of the human small intestine.[231] Rats lack a sigmoid colon, gallbladder, and cystic ducts, while pig colons are larger than those of humans.[232-234]

Beyond anatomical differences, behavioural disparities impact the relevance of these animal models. Rats typically consume small, frequent meals, whereas humans eat larger, less frequent meals.[235] Pigs, on the other hand, consume more food relative to their body weight than humans do.[236]

Laboratory conditions can further influence the study of GI diseases. In a 2024 study, researchers found that the temperature at which mice are housed within a laboratory can significantly affect their gut motility and microbiota.[237] The source of the animals can also lead to variations in gut microbiomes due to differing environmental factors.[238] Species-specific microbiome differences play another role: Pigs have little Bifidobacterium, a major species in the human gut.[239] Given the role of gut microbiota in immune response, these differences may significantly impact study outcomes.[240]

Animal models of human GI conditions are criticized for their poor predictive value regarding disease outcomes and clinical efficacy in humans, especially for conditions like irritable bowel syndrome (IBS) and irritable bowel diseases (IBD), the pathogenesis of which remains not fully understood.[241]

IBS is a chronic condition affecting the lower GI tract. Fifteen percent of adults in the U.S. experience IBS symptoms, which include abdominal pain accompanied by diarrhoea, constipation, or both.[242] While the exact cause of IBS remains unclear, it is believed to involve a combination of physical and psychological factors, particularly stress and anxiety,[243] which cannot be faithfully simulated in nonhuman models.

Animal models of IBS are typically created by subjecting animals to stress during early development.[244] These models have significant limitations, such as their inability to replicate the constipation or mixed bowel responses of human patients. Additionally, human IBS patients often present with overlapping disorders, such as bladder pain syndrome, chronic pelvic pain, anxiety, and depression—none of which are modelled in experiments on animals. Behavioural changes, such as anxiety or depression, are difficult, if not impossible, to measure in animals (see the appendix on Neuropsychiatric Disorders and Neurodivergence). Most experiments use male animals, despite IBS being more commonly diagnosed in females. Additionally, abdominal pain, the primary symptom of IBS, cannot be accurately assessed in animals, as there is no measurable phenotype specific to the visceral pain experienced by humans. These shortcomings make IBS experiments on animals inappropriate for understanding IBS pathophysiology and developing effective treatments.[245]

IBDs, which include ulcerative colitis and Crohn's disease, are chronic inflammatory conditions often affecting the large and small intestines. IBDs impact two to three million people in the U.S.[246,247] IBD patients suffer from rectal bleeding, severe diarrhoea, abdominal pain, fever, and weight loss. The causes of IBDs are believed to involve a combination of genetic, immune, microbial, and environmental factors, though the precise mechanisms are not fully understood.[248]

In IBD research, scientists induce colitis by administering irritating substances or using genetically engineered mice. However, reproducibility remains a significant issue. Different mice strains exhibit varying susceptibilities to chemically induced colitis, and microbiome differences across strains or vendors can also influence disease development in genetically engineered mice. Given that both genetic and environmental factors contribute to IBD, an animal model that lacks these human-specific characteristics cannot effectively replicate these diseases. For example, genetically engineered mice are often created by mutating a single gene, but human IBDs are polygenic.[249] Furthermore, chemically induced colitis in mice typically results in acute injury over a few days, whereas IBDs in humans develop over years.[250]

A key example of the limitations of animal models is IL-17 inhibition, which effectively treats colitis in mice but has failed in Crohn's disease patients, sometimes even worsening the condition.[251,252] A 2019 review noted that "while there are many in vivo models of IBD, none adequately predicts response to therapeutics."[253] The disappointment of IL-17 inhibition in clinical trials illustrates how a treatment that works in animal models can fail in humans. Conversely, some therapeutics that show promise for treating IBDs in patients have failed in mouse models.[254,255]

Given these limitations, it is clear that no animal model can accurately replicate human GI disorders. These conditions are influenced by a complex interplay of environmental, genetic, and microbial factors that cannot be fully captured in artificially induced animal models. Therefore, prioritizing human-relevant research methods, such as organoids, microfluidics, and organ-on-a-chip technologies, is crucial. Recent developments in this area include the following:

- Biological engineers at MIT created a human multi-organ model of ulcerative colitis to study its impact on the gut-liver-immune axis.[256]

- Scientists at the Francis Crick Institute, in collaboration with UCL and Imperial College London, used a multi-omics approach to identify a new biological pathway related to IBDs, finding the gene ETS2, which is linked to higher IBD risk.[257]
- A group of researchers and physicians in Missouri and North Carolina created a neonatal-intestine-on-a-chip to study necrotizing enterocolitis, a deadly GI disease seen in premature infants. They successfully showed that this model can recapitulate disease pathology and plan to use this method for therapeutic testing.[258]
- Physicians and scientists in Boston obtained biopsies, blood, and stool samples from patients at Cincinnati Children's Hospital, Massachusetts General Hospital, Emory University Hospital, and Cedars-Sinai Medical Center to create a longitudinal molecular profile of their microbiomes. Using a multi-omics approach, they were able to identify microbial, biochemical, and host factors involved in IBD-induced dysregulation.[259]
- Researchers and physicians in Houston used patient-derived intestinal organoids to explore the link between telomere dysfunction and IBDs, suggesting that addressing telomeric dysfunction could be a therapeutic strategy.[260]

The anatomical and physiological differences between nonhuman and human GI systems, coupled with the artificial induction of GI diseases in animals, hinder reliable study outcomes. Furthermore, many of these induction methods involve invasive and painful procedures, leaving the animals in distress until they are killed.[261-266] Given that animal models of GI diseases do not reliably reflect human pathology and contribute to animal suffering, it is essential to transition toward the numerous non-animal methods using human tissues or consenting patients.

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Nerve Regeneration

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: "[D]ifferences in injury type between laboratory-induced SCI and clinical SCI, difficulties in interpreting functional outcome in animals, and inter-species and inter-strain differences in pathophysiology of SCI."[267] In a systematic review of the use of animal models to study nerve regeneration in tissue-engineered scaffolds, researchers have said that most "biomaterials used in animal models have not progressed for approval to be tested in clinical trials despite the almost uniform benefit described in the experimental papers."[268] The authors lamented the low quality of described experiments on animals, as 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% reported beneficial results, 58% reported no effect, and 8% had mixed findings.[269] The results were inconsistent 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. They suggested that as a result of these immutable inter- and intra-species differences, no human-relevant animal model can be developed, concluding that the "research emphasis should be on the development and use of validated human-based methods."[270]

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.*[271]

More specifically, the inconsistencies between animal models and the clinical situation are significant[272] and 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.*[273]

To induce a spinal cord injury in animal models, experimenters use physical force to damage the spinal cord. There are many different methods, such as contusion, which involves displacing the spinal cord by dropping a weight, or distraction, which applies a traction force to stretch the spinal cord. Regardless of the method used, achieving consistency and reproducibility is challenging due to the inability to replicate the same spinal cord injury every time they perform the procedure. For example, in contusion-induced injuries, variability can arise from the rod bouncing after it hits the spinal cord, potentially causing multiple impacts.[274]

In addition to consistency issues, many of these models do not accurately reflect the mechanisms of SCI in humans. A compression model created using forceps does not replicate the acute impact seen in most human SCI, and the devices used for the distraction model often induce injury too slowly to emulate human injury. Chemically induced SCI is employed to study secondary injuries associated with SCI, usually involving the injection or application of a toxic chemical to the area of interest. However, challenges with chemically induced SCI include ensuring accurate delivery of the chemical to the correct region of the spine.[275]

Biomedical engineers have noted that researchers "are incapable of truly mimicking human neural injuries in animal models because of the extensive anatomical, functional, molecular, immunological, and pathological differences between humans and frequently studied animals."[276] Human-relevant methods 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 human cells. Ex vivo models, such as those using three-dimensional engineered scaffolds, bioreactors, neurospheres, and organoids, allow for more controlled studies on specific parameters than animal experiments.[277] Bioprinting can use bioinks containing human cells and materials to construct heterogeneous tissue models in a single step and with remarkable consistency,[278] an aspect of nerve regeneration research that has been notably lacking in animal models.[279]

Engineers and researchers at the University of Pittsburgh Medical Center and Carnegie Mellon University have emulated mild and moderate traumatic brain injury (TBI) using human cerebral organoids. Their study identified important genetic repercussions of TBI on the brain that can be used to diagnose the condition and create personalized treatments for patients.[280] Neuroscientists have engineered human spinal cord organoids that display functional neuronal activity and hold promise for investigating SCI therapies.[281]

Microfluidic devices are "adaptable for modelling 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."[282] For example, brain-on-chip platforms offer a promising avenue for personalized medicine, as a patient's own cells can be used to create a custom device to investigate treatment options.[283] Axons-on-a-chip can model diffuse axonal injury, allowing researchers to track the intracellular changes immediately following injury and offering a platform for screening treatments.[284] These systems offer advantages in precision, scalability, and cost-effectiveness when compared to traditional cell culture or experiments on animals, and are currently on the market and available for neural regenerative medicine research.[285]

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Neurodegenerative Diseases

There is sufficient literature documenting the failings of various animal models of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). While a lengthy appendix could be written for each disease, many of the same limitations of animal models prohibit translation across these conditions, and they will be discussed briefly as a whole.

All these diseases are human-specific, meaning they do not occur naturally in other animals. No animal model has been developed that recapitulates all aspects of a particular neurodegenerative disease.[286] For AD research, the clinical failure rate for new drugs was last estimated to be 99.6%,[287,288] and recent monoclonal drugs approved for AD have been controversial due to adverse effects and questionable efficacy.[289,290]

A bioinformatics analysis comparing the transcriptional signatures of AD, PD, HD, and ALS with mouse models of these diseases produced 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.*[291]

These molecular discrepancies underscore the artificial methods used to create such models. Physical and chemical lesioning or systemic administration of toxins are commonly 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 disease models, such as the 6-OHDA and MPTP animal models of PD and the 3-NP animal model of HD, fail to capture the progressive nature of the disorders they aim to mimic. In addition, scientists often use young animals to "model" diseases associated with aging,[292] further reducing their relevance. For example, "[c]ommonly used AD mouse models, like the 5xFAD, display amyloid deposits starting at 2–4 months of age…this early accumulation can be translated to Aβ deposits occurring in 4–8 year-old humans, a scenario not found even in the most aggressive cases"[293] of AD.

Genetically modified mouse models exhibit inconsistent pathological and behavioural phenotypes, partly due to variations in transgenes used, inconsistencies in transgene insertion and expression, and differences in mouse background strains.[294] As of 2024, 210 transgenic rodent AD models have been developed.[295] In a review on the relevance and translational validity of these mouse models, researchers described their shortcomings:

> *Some transgenic models can present a very aggressive disease phenotype compared to the human form of the disease…while others fail to demonstrate aspects of neuronal loss and dysfunction… Of additional concern is the fact that mouse models often fail to show a substantive neuronal loss even in the presence of amyloid deposits and generate amyloid peptides different from those found in human brain... In some instances, the failures encountered with animal transgene models reflect the fact that they are based on intrinsically flawed hypotheses and the constructs used to interrogate these; in other instances, they reflect a lack of diligence on the part of investigators to ensure best practices in the husbandry and use of these models. Despite their limitations, these flawed models become widely utilized, with their relevance being overstated because of the lack of any viable alternatives, while only lip-service is paid to their validity as they become de rigor and self-perpetuating—driving the field down a blind alley.*[296]

Fundamental genetic differences further hinder translation. For example, "knock-in models require the presence of multiple APP [amyloid precursor protein] mutations not found in humans," murine tau differs structurally from human tau, and "key amino acid substitutions make murine Aβ less prone to aggregation when compared to its human counterpart."[297] These differences make animal models of neurodegenerative disease misleading and waste precious time: A genetic target for AD research previously identified as upregulated in mouse models was, unsurprisingly, not found to be upregulated in humans in a recent postmortem study.[298] For PD, nonhuman primate studies do not "constitute a valid scientific modality for the complete understanding of PD and for the development of future neuromodulation therapeutic strategies."[299]

As in much of biomedical research, animals suffer greatly when used to mimic neurodegenerative diseases. In an analysis of published research 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."[300] 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 adhering to the 3Rs principles and compromising not only animal welfare but also the relevance of their studies to HD.[301]

As animal studies fall short, scientists and policymakers are increasingly recognizing the need for human-relevant research strategies. Following a review of AD research, an interdisciplinary panel recommended reallocating funding away from animal studies and toward more promising techniques, such as patient-derived hiPSC models, "omic" technology (genomics, proteomics, etc.), in silico models, neuroimaging, and epidemiological studies.[302]

The following are highlights in recent cutting-edge, human-relevant neurodegenerative disease research.

- At Brigham and Women's Hospital, researchers differentiated hiPSCs into neurons that quickly develop protein inclusions mimicking those found in the brains of individuals who died with inclusionopathies. Using this method, the team created more than 60 human cellular models that other laboratories can use to study human neurodegenerative diseases.[303]
- A team of scientists at Washington University in St. Louis used cells from patients with AD to develop a relevant, 3D human cellular model for late-onset AD (which accounts for 95% of cases). This model allows for the study of age-associated neurodegeneration.[304] Another team conducted a proteomic study on the cerebral spinal fluid of patients with AD to identify biomarkers that can be detected decades before symptoms arise.[305]
- Researchers at the Barcelona Institute of Science and Technology developed an organ-on-a-chip to evaluate the brain permeability of nanotherapeutics and facilitate personalized research and therapy for AD.[306]
- At the Vienna BioCenter, scientists created an in vitro model of the human dopaminergic system with ventral midbrain–striatum–cortex assembloids to improve the study of PD cell therapies.[307]
- Researchers at the University of Luxembourg used human organoids and assembloids—including those developed with patients' own cells—to understand the early stages of PD and factors influencing susceptibility.[308,309]
- Boston-based Emulate, Inc. engineered a human brain-on-a-chip that represents areas affected by PD, reproduced features of the disease, and can be used to identify and test new therapeutic targets.[310]
- Scientists in Germany used human brain organoids to identify a gene implicated in HD that may damage the brain before symptoms arise and could serve as a focus for drug development. Restoring the function of this gene reversed the HD phenotype.[311]
- University of Central Florida scientists used cells from patients with ALS to develop a disease-specific neuromuscular junction-on-a-chip and tested the effects of a compound on clinically relevant functional measures of ALS.[312]
- In another patient-specific study, a team at Utrecht University used human brain organoids to improve the understanding of synaptic changes in ALS patients before the onset of symptoms.[313]

For decades, experimenters have tormented monkeys, mice, dogs, and other animals in an attempt to model these devastating diseases. However, since other animals don't develop these human neurodegenerative diseases naturally, experimenters have manipulated their genomes to force discrete symptoms. The results, after decades of tests, include more than 100 failed drugs, an untold number of animal deaths, and the continued suffering of humans living with these conditions. For these patients, a shift to human-relevant methods is long overdue.

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Neuropsychiatric Disorders and Neurodivergence

Like many other animal models of human disease, animal models used in an attempt to study human neuropsychiatric disorders and neurodivergence lack critical aspects of model validity. These deficiencies include (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; (2) face validity, meaning that animals cannot "recapitulate important anatomical, biochemical, neuropathological, or behavioural features of a human disease;"[314] and (3) predictive validity, meaning that results from experiments on animals fail to translate into similar results in humans reliably.

No single animal model replicates all aspects of a human neuropsychiatric condition, and features of human behaviours that represent hallmarks of these disorders cannot be accurately produced or assessed adequately in animals.

For example, human depressive disorders are characterized in part by feelings 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 an experimenter places a rat or mouse in a container of water with no way to escape or rest. Experimenters falsely interpret the amount of time the animal spends swimming or struggling to escape as a measure of the animal's lack of despair. This misguided notion originated from the observation that swimming and struggling time could be extended by giving the animal some types of human antidepressants (even though this assumption ignores the many false positives and false negatives that the test produces). As has been widely discussed in the scientific literature, an animal's behaviour in the forced swim test may represent an evolutionary adaptation to the stressful situation and should not be used to determine their mood.[315] The results can be influenced by an animal's strain and many experimental variances, including water depth, container dimensions, and temperature.[316-319]

A PETA neuroscientist and collaborators have published papers discrediting the use of the forced swim test as a valid method for screening antidepressant drugs. Their findings revealed that the use of this test by the world's top 15 pharmaceutical companies did not produce any drugs currently approved for treating depression in humans.[320] They also highlighted actionable steps regulatory authorities could take to eliminate the use of the forced swim test (and the similar tail suspension test) in the pharmaceutical industry.[321]

Other animal behavioural tests—such as the sucrose preference test (for anhedonia),[322-324] open field test and elevated mazes (for anxiety),[325,326] marble burying (for compulsion),[327] chronic unpredictable stress (to induce psychopathologies)[328]—have similar flaws. These concerns have led to the awareness that "some of these assays must be discontinued, and placed in the past; while we seek improved, innovative strategies for outcome measures."[329]

A series of citation analyses demonstrated that researchers studying major depressive disorder in humans rarely cite results from experiments on rats or monkeys, two of the most commonly used species in this field. Instead, they more frequently relied on research results using human cells and human biological data.[330-332] A similar failure of animal studies to contribute to clinical knowledge has been noted in bipolar depression research,[333] and animal studies have been cited as the primary source of attrition (failure of drugs) in neurobehavioral clinical trials.[334] Despite these warnings, thousands of researchers have continued to use flawed assays like the forced swim test to draw erroneous conclusions about an animal's mood[335] or the potential effects of compounds on human depressive disorders.[336]

Significant physiological differences between humans and other animals contribute to the low translation rate. For example, the gene encoding tyrosine hydroxylase, the enzyme involved in dopamine formation, is regulated differently in humans than in mice.[337] Misregulation of tyrosine hydroxylase has been implicated in

several psychiatric illnesses, such as bipolar disorder and schizophrenia. In a 2019 study published in Nature, 64 researchers analysed the brains of mice and humans and found substantial species differences in types of brain cells and how they produce proteins critical to neuropsychiatric function. The authors noted numerous "failures in the use of [the] mouse for preclinical studies" because of "so many [species] differences in the cellular patterning of genes."[338] Rodents and humans also diverge in other critical areas for neuropsychiatric research, including the diversity, organization, and volume of neuronal cell types; relevant neural circuitry; volume of neurotransmitters available in specific cell types; and neurotransmitter receptor availability and kinetics.[339]

Beyond the lack of applicability, animal neuropsychiatric models cause immense suffering. To induce "depression," experimenters subject animals to uncontrollable pain through electric shocks or chronic stressors, such as restraining them for extended periods, 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 other 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-like or altered mental state. In this field in particular, "animals are likely undergoing experimental procedures that do not provide the epistemic benefit we are sacrificing them for."[340]

Funds should be redirected from the use of animals toward relevant, human-based experimental methods, including the following:

- Human brain organoids: Advanced, 3D in vitro cultures of human brain cells that replicate the cellular organization and signalling of human brain tissue. These have been used to study mood disorders, psychoses, and neurodivergence.[341-344] Organoids can be combined to form self-organizing assembloids that mimic complex interactions between different parts of the brain,[345,346] such as the cortico-striatal-thalamic-cortical circuit and thalamocortical assembloids recently developed by a team at Stanford University to study human neurodevelopmental conditions like autism, Tourette syndrome, and schizophrenia.[347,348] Researchers at the University of California San Diego and the University of Massachusetts at Amherst are developing disease-specific brain organoids using cells from patients with genetic mutations linked to neuropsychiatric disorders for therapeutic applications.[349-351]
- Omics research: This is being applied to better understand the underpinnings of human neuropsychiatric conditions. The PsychENCODE Consortium, a collaboration of multidisciplinary teams, uses state-of-the-art methods to create large datasets from human postmortem brain samples.[352] Some teams are analysing existing data to characterize gene variants related to these disorders.[353]
- Brain imaging: Techniques including magnetoencephalography, high-density electroencephalography, magnetic resonance spectroscopy, transport-based morphometry, and functional magnetic resonance imaging—often combined with machine learning and genomics—are being used to study human psychiatric conditions and neurodivergence directly in individuals with lived experience.[354-358]
- Longitudinal studies: Tracking individuals over extended periods provides insights into the effects of environmental stimuli, medical history, and life events on the incidence and progression of neurodevelopmental conditions.[359,360]
- In silico clinical trials: Virtual patient models have been used to evaluate the potential of drugs for conditions like attention-deficit/hyperactivity disorder and schizophrenia.[361,362]

Given the psychological distress inflicted on animals and the inapplicability of the results to humans, the use of animals in human neuropsychiatric and neurodivergence experiments should end. Resources must be diverted to human biology-based research like the examples listed above.

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Pandemic Preparedness

To say that the COVID-19 pandemic changed life as we know it is an understatement. However, a silver lining may be its potential to lead to an entirely new era of biomedical research and vaccine development. To accelerate COVID-19 vaccine development, both the FDA and NIH greenlighted landmark human clinical vaccine trials without requiring extensive tests on animals beforehand. Instead, the human and animal testing proceeded in parallel,[363] a change that PETA urged the FDA to extend to all new drugs in development (e-mail communication, May 5, 2020, https://www.peta.org/wp-content/uploads/2020/05/2020.05.05-FDA-CommissionerCOVID-19-letter-FINAL.pdf).

Although time constraint was an obvious factor in this decision, it is essential to note that many species do not respond to SARS-CoV-2 infection in the same way humans do. When *The New York Times* asked about seemingly promising experimental results in rhesus macaques, Dr Malcolm Martin, a virologist at the NIH, "cautioned that monkeys are different from humans in important ways."[364] The interviewer noted that "[t]he unvaccinated monkeys in [the vaccine experiment] didn't develop any of the severe symptoms that some people get following a coronavirus infection" and quoted Martin as saying, "It looks like they got a cold."[365] Even genetically engineered mice, who are made susceptible to the disease, only show mild symptoms. "Humanized" mice (those who are engineered to express human immune factors) do not solve this problem, as "many human factors cross-react with murine cells, which may lead to unexpected phenotypic changes."[366]

Amid the COVID-19 pandemic and outbreaks of other infectious diseases like H5N1, it has become increasingly clear that infectious disease research and pandemic preparedness should be prioritized. Human-relevant research can lead the way.

Many scientists are using innovative non-animal methods to study existing pathogens and those with pandemic potential. These methods include human lung and intestinal organoids, three-dimensional reconstructed human respiratory tissue models, human oral tissue samples from healthy volunteers, advanced computer simulation and supercomputers, human genetic analyses, human challenge studies, human-derived antibodies, and human organs–on-chips modelling human lungs, mouths, eyes, noses, and intestines. Complex *in vitro* human models, such as organoids and organs-on-chips, are expected to be particularly valuable for infectious disease research and developing vaccines and antiviral drugs.[367-371] Here are a few recent examples:

- Human lung and brain organoids are being used to study SARS-CoV-2 infection mechanisms, test potential therapies, and investigate the virus' effects on the brains of healthy individuals and those with comorbidities.[372-376]
- Researchers in Japan created patient-specific livers-on-chips to explore SARS-CoV-2-induced liver dysfunction and to evaluate drugs to treat it.[377]
- Using cells isolated from human lung tissue, researchers engineered human lung organoids to study H5N1 virus replication, host cell survival, and lung immune responses to different viral strains.[378]
- According to a recent review, "microphysiological systems and organoids are already used in the pharmaceutical R&D pipeline because they are prefigured to overcome the translational gap between

model systems and clinical studies."[379] The authors explain that complex, human-derived systems like organoids and microphysiological systems will be essential for research on filovirus and bornavirus infection in humans, for which "animal models cannot capture the respective pathogenesis and disease in full."[380]

- Respiratory syncytial virus is being studied using *ex vivo* samples from patients to determine why some have a more severe reaction to the infection[381] and with human airway organoids to develop and test antibody therapies.[382]
- Individuals with post-infectious disease syndromes like long-COVID and myalgic encephalomyelitis/chronic fatigue syndrome have been studied using brain imaging; analyses of skin biopsies, blood, and cerebrospinal fluid; monitoring of diet, sleep, and cardiac measures; and more to phenotype these conditions, understand how they occur, and guide potential therapies.[383]
- *In silico* tools have been used in drug repurposing studies to identify existing therapies that could treat COVID-19.[384]

In addition to adopting non-animal methods to study and develop treatments, it's even more critical to take measures to prevent the spread of emerging pathogens. Ending the importation of wild species into laboratories for experimentation is a key step. Long-tailed and rhesus macaques are the most commonly used nonhuman primates in experimentation, the most commonly traded primate species, and the species that harbours the highest volume of potential zoonotic disease.[385,386] While primate suppliers and buyers claim to support efforts to reduce the use of wild-caught macaques in research, investigations have revealed that international suppliers have falsely labelled wild-caught macaques as captive-bred and sold them to laboratories.[387] This practice risks disease spillover and compromises the results of experiments conducted on these animals, whose health histories are unknown.

Macaques are often captured and imported from regions endemic for melioidosis, a life-threatening illness caused by *Burkholderia pseudomallei*. Though the Centers for Disease Control and Prevention (CDC) requires that monkeys imported from these regions undergo a mandatory quarantine, *Burkholderia pseudomallei* can remain dormant for long periods, and animals have been released into laboratories while still infected.[388] Macaques have also been imported while harbouring tuberculosis-causing mycobacteria.[389,390] According to the CDC, "In the United States, there is no centralized system for reporting TB in NHP that are not in CDC-mandated quarantine (minimum of 31 days after importation). Therefore, it is unknown how common TB is in NHP in the United States."[391]

Ending the global trade of monkeys for experimentation would eliminate a major risk factor in zoonotic disease spillover, reduce the dissemination of unreliable data collected from animals of unknown origin, and stimulate the move toward human-relevant research methods. This is a critical step in protecting public health and preventing the next pandemic.

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Stroke

Stroke, a serious condition affecting the brain's blood vessels, is the fifth leading cause of death and a major contributor to disability in the U.S.[392] It occurs when blood flow to the brain is interrupted, either by a clot (ischemic stroke) or a burst blood vessel (haemorrhagic stroke), resulting in damage and death of brain cells due to lack of oxygen. After an ischemic stroke, recanalization (restoration of blood flow to the brain) is the only immediate treatment available in the acute phase.[393] Procedural intervention by endovascular therapy is the standard treatment for ischemic stroke when possible, but is only effective in approximately 25% of cases.[394]

Despite over a thousand neuroprotective drugs showing promise in animal models, none have translated into effective human therapies for stroke.[395] Our understanding of the biological processes driving human stroke recovery remains limited,[396] and developing accurate models of the central nervous system is challenging due to the complexity of the human brain. Current animal models, which primarily use rats, lack essential human characteristics, differ in stroke recovery compared to humans, and raise ethical concerns.[397,398] For example, ischemic stroke typically occurs in elderly patients with comorbidities, whereas experiments are predominantly carried out in young, healthy animals who often exhibit spontaneous recovery.[399]

Significant differences in brain composition—such as white matter making up 60% of the human brain but only 10% of the mouse brain[400]—and variations in blood-brain barrier physiology[401,402] play crucial roles in stroke pathology. Additionally, differences in clot composition, neuronal function, and inflammatory processes among species further contribute to the poor translatability of animal models in stroke research.[403-405]

A 2010 analysis of 16 systematic reviews (including 525 different studies) on human stroke interventions tested in animal models revealed that the efficacy of these experiments on animals was overstated by approximately one-third due to publication bias (the propensity of researchers and journals to publish results showing positive outcomes and omit studies with negative or null data).[406] The authors noted that "participants in clinical trials may be put at unnecessary risk if efficacy in animals has been overstated."[407]

In silico modelling shows potential to replace animal experimentation in stroke research. Projects like INSIST (IN-Silico trials for treatment of acute Ischemic STroke) use virtual patients to simulate stroke treatments, replicating clinical characteristics, such as clot properties, vessel geometries, and patient medical records.[408] These models, which allow for virtual drug testing and the detailed study of thrombosis and brain perfusion in humans, "have the potential to lead to a more effective human clinical trial design, reduce animal testing, lower development costs, and shorten time to market for new medical products."[409] A groundbreaking *in silico* trial published in 2021 predicted aneurysm treatment responses using 164 virtual patients with 82 unique anatomies.[410] This model outperformed experiments on animals, identifying new risk factors for treatment failure in days instead of decades. Virtual modelling can also assist patient-tailored clinical decisions for stroke and other neurological conditions. However, regulatory reform for *in silico* trials is urgently needed to advance the field.[411]

Researchers are also exploring new technologies and cell-based methods to enhance recovery by replacing damaged brain tissue with stem cells.5 Recently, stem cell therapy using patients' bone marrow or allogeneic

umbilical cord blood has shown improved neurological outcomes in clinical trials.[412-415] In preclinical research, the isolation of human stem cells and hiPSCs has advanced the development of scalable human models in neurobiology.[416,417] Innovative 3D systems, like organs-on-chips and brain organoids,[418,419] may mimic complex cell interactions and *in vivo* physiology better than animal models, while 3D printing[420] enables the creation of detailed nervous system models for preclinical drug testing and clinical applications.

Accurately modelling ischemic responses requires understanding cellular interactions that influence blood-brain barrier permeability, cerebral oedema, and neurovascular responses under pathological conditions. Because these interactions ultimately affect stroke outcomes, it is essential to create realistic models. Combining hiPSCs with advanced cell culture technologies has allowed replicating specific human nervous system features. For example, Kook and colleagues developed a vascularized model by coculturing vascular and cerebral spheroids generated by hiPSCs.[421] In another brain organoid study, Xu et al observed morphological and synaptic changes in microglia cells after viral exposure.[422] Additionally, microfluidic models enable the use of patient cells and real-time monitoring of human brain dynamics, such as blood-brain barrier permeability and shear stress, which are not feasible in experiments using other species. *Ex vivo* brain slices are another valuable method for studying human brain tissue, as they preserve *in vivo* properties, spatial organization, and complex networks of various cell types.[423]

In recent years, *in vitro* systems for studying stroke and the human nervous system have advanced significantly, becoming sought-after tools for studying human brain function and improving stroke treatment strategies.[424] Now that these tools are available, researchers must adopt them, and funders must support their uptake.

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Substance Use Disorder

Fundamental aspects of nonhuman animals make them inappropriate for the study of substance use disorders (SUD). First, the use of and dependence on drugs in humans is a vastly complex experience, one that has been impossible to mimic using animals in a laboratory setting.[425] It has been argued that attempts to model SUD in nonhuman 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."[426]

> *[A]nimal models cannot capture many key aspects of human brain disorders that may be caused by an SUD, which often involve the interplay of genetic, developmental, and environmental factors…In addition, studying the brain in live animals involves invasive techniques that can affect the health and behaviour of the subjects, potentially confounding results…Consequently, it's hard to translate research outcomes from animal models into effective clinical treatments for SUDs due to the inter-species differences in neuro systems between human and animal models.*[427]

Several diagnostic criteria for SUD are impossible to model in animals since they require an individual to self-report. These include "(i) subjective craving, (ii) taking the substance in larger amounts or for longer than intended and (iii) wanting to cease or reduce substance use but being unable to."[428]

Second, the pharmacokinetic actions of drugs differ among species. For example, "the rate of metabolism of MDMA and its major metabolites is slower in humans than rats or monkeys, potentially allowing endogenous neuroprotective mechanisms to function in a species-specific manner."[429] 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.[430] Since MDMA is being explored not only because of its use as a recreational drug but also for its potential therapeutic use, accurate knowledge regarding its safety in humans is paramount.

Third, serious flaws in the experimental design of substance use experiments on animals skew the interpretation of their results. Unlike humans, whose experience with SUD is primarily shaped by individual choice to consume an addictive substance—often over other rewarding alternatives —animals in laboratories are typically not given this option. When they are, the majority will choose an alternative reward, such as sugar, over the drug.[431] This holds for primates as well as mice and rats. Even among animals with a history of heavy drug use, only about 10% continue to self-administer the drug when presented with another rewarding choice.[432] In a review on the "validation crisis" in animal models of drug addiction, it has been said that the lack of choice offered to animals in these experiments raises "serious doubt" about "the interpretation of drug use in experimental animals."[433]

The nonhuman animal has been called a "most reluctant collaborator" in studying alcohol use disorder and exhibits a "determined sobriety," which the experimenter must fight against to overcome "their consistent failure to replicate the volitional consumption of ethanol to the point of physical dependency."[434] 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."[435]

Despite the epidemic of drug dependence and overdose in the U.S. and the prevalence of SUD research conducted on animals, there are only limited treatment options available for individuals addicted to opioids, nicotine, and alcohol, and no approved treatments for marijuana, stimulant, or polysubstance users.[436] Leadership at the National Institute on Drug Abuse has noted that pharmaceutical companies show little interest in investing in treatments for SUD due to the stigma and complexities of the disease.[437,438] While data from animal studies were once hailed as promising in certain drug classes and relapse prevention, most have either failed to be effective in human trials or were not tolerated well by humans.[439,440] Some researchers argue

that "these failures illustrate the inability of animal models to capture the complex nature of addiction and its treatment" and that "findings from animal models of addiction have generated a misleading picture of the nature of addictive behaviour in humans."[441]

Non-invasive human and human biology-based research methods are now providing answers to questions that nonhuman animals are fundamentally unable to solve. Rutgers University Robert Wood Johnson Medical School researchers authored a review article describing how hiPSCs 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."[442]

Forward-thinking scientists around the world are carrying out human-relevant, non-animal research on SUD:

- Researchers are using postmortem human samples to model changes in the brain and brain cells induced by SUD. For example, at the University of Texas Health Science Center and Baylor College of Medicine, researchers engineered a novel hiPSC model of neural progenitor cells and neurons from postmortem human skin cells, directly comparing the new models to brain tissue from the same donors to model opioid-induced brain changes.[443] Heidelberg University scientists conducted an epigenomics study on postmortem brain tissue from individuals with cocaine use disorder to understand how the disorder alters synaptic signalling and neuroplasticity.[444]
- A recent University of Pennsylvania study used 3D genomic datasets to sequence more than 50 diverse human cell types to identify genetic and cell targets that underlie SUD.[445]
- A multi-omics study conducted by a team of researchers across the U.S. as part of the Million Veteran Program used systems biology to reveal key genetic targets for new drugs to treat opioid use disorder.[446]
- University of Central Florida researchers have developed a hiPSC model for studying opioid use disorder and opioid-induced respiratory depression to combat the opioid overdose crisis.[447]
- At North Carolina State University, scientists co-cultured human neurons to form assembloids used to understand single-cell human molecular responses to cocaine and morphine.[448] Human-derived assembloids and organoids "show unique potential in recapitulating the response of a developing human brain to substances"[449] and will also be helpful in studying *in utero* exposure to drugs of abuse.
- Research on better ways to treat human pain is crucial for reducing opioid use disorder incidence and relapse. Researchers at Queen's University Belfast used *in vitro* and *in vivo* human peripheral nerves.[450] Biotechnology companies like AxoSim, NETRI, and others have developed human neuronal in vitro models that can be used for human pain research.

In addition, the funds currently supporting ineffective and wasteful SUD studies in animals could redirected to support effective drug prevention, rehabilitation, and mental health programs.

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Women's health

While women face significant health risks independent of sex or gender, many health outcomes are closely linked to the reproductive cycle and vary throughout the lifespan.[451] Historically underfunded and understudied, women's health issues such as infertility, endometriosis, adenomyosis, and menopausal symptoms require urgent attention.[452]

A significant obstacle to using other species to study women's health is the anatomy of the reproductive tract. For example, mice have a closed reproductive system with tightly coiled oviducts opening into the bursal space.

In contrast, the human reproductive system is open to the peritoneal cavity. This allows endometrial cells, shed during menstruation, to flow backward (retrograde menstruation) into the peritoneal cavity. This retrograde menstruation is linked to the development and symptoms of endometriosis. "[F]rom a morphogenetic perspective Müllerian duct development differs considerably in mice and humans,"[453] resulting in the development of fallopian tubes in humans and the Müllerian vagina in mice.

Endometriosis and adenomyosis are closely related gynaecological conditions that cause pelvic pain, miscarriage, and infertility and affect around 10% of women.[454-456] Despite being first described centuries ago, significant gaps in the diagnosis and treatment of these conditions are due to the incomplete understanding of underlying mechanisms[457] that have been repeatedly investigated using failed animal models.

Human endometriotic lesions, which are not yet fully characterized, vary significantly in location, size, colour, and depth.[458] Additionally, endometriotic lesions have distinct aetiologies that are impossible to fully replicate in animal models, requiring invasive methods such as surgical engraftment, intraperitoneal injection, or direct tissue injection into the endometrium.[459,460] These artificial approaches often result in cellular contamination with non-uterine tissue and local inflammation in animals.[461] Transgenic *de novo* mouse models rarely succeed in replicating endometriosis due to the lethal phenotypes often associated with knocking out essential genes.[462] In addition, the long latency period required for endometriosis to develop—something unachievable with short-lived species like mice—underscores the fundamental limitations of animal models.

The process of menopause and its symptoms vary widely among women, primarily influenced by factors such as the remaining number of eggs in the ovaries, lifestyle, diet, and ethnicity.[463-465]During the menopause transition, fluctuations in oestradiol levels in the perimenopausal phase can cause specific, complex, and protracted physiological, behavioural, and neurological changes[466] that experiments on animals fundamentally fail to replicate.

The oestrous cycle of other primates and rodents differs considerably from that of humans.[467] The vast majority of nonhuman animals do not experience menopause, and their fertility patterns differ significantly from those of humans. Fertility decline can occur in mice as early as 8 months,[468] or about one-sixth of their potential lifespan. The menstrual cycle of other primates and rodents differs in length, hormone fluctuation, and the ways in which these hormones regulate the hypothalamic-pituitary-gonadal axis compared to humans.[469-471]

Given the many biological challenges described above, researchers attempt to replicate menopause and uterine lesions in animals using unnatural methods. Ovariectomy—the surgical removal of ovaries—is considered the gold standard for creating these symptoms in animals, but the procedure is an invasive and clinically irrelevant method for inducing menopause. Menopause is a gradual transition—not an abrupt event—and animals do not experience the same symptoms as humans, such as brain fog or the continued release of androgens by the ovaries.[472] Other animal models created by the chemical induction of premature ovarian failure are prone to experimental confounds, such as discrepancies related to the dose and duration of the treatment, the development of unrelated neurological issues,[473] and the inability to model responses to drugs that may reverse premature ovarian failure in humans.[474]

Most experiments use young animals, such as young marmosets, whose physiology drastically differs from the aging humans they aim to mimic. Genetic patterns in the brains of these animals don't align with humans in the menopausal transition, meaning cognitive decline caused by oestrogen fluctuation and loss during this period cannot be replicated.[475]

To design more effective interventions, it is essential to deepen the understanding of human-specific biological mechanisms that affect women's health and fund the tools necessary for this this critical yet often overlooked research.

Collective efforts for phenotypic characterization and biobanking of human endometrial lesions,[476,477] combined with machine learning tools that analyse patient data and wearable devices to identify potential risk factors, can produce data that has been historically difficult to replicate using simpler *in vitro* models. In one study, researchers developed a unified predictive model for the diagnosis of endometriosis using a dataset of over 5,000 women.[478] The model analysed more than 1,000 variables, including lifestyle, genetic variants, and medical history, and identified year of birth and irritable bowel syndrome as significant risk factors.

The limitations of experiments on animals and traditional *in vitro* models have driven the development of advanced microfluidics platforms that accurately recapitulate the human reproductive system.[479] These include the human placenta-on-a-chip, which allows for the study of maternal-foetal interface and pregnancy-related conditions,[480-482] and standardized hiPSC protocols.[483] Another vascularized multicellular model effectively mimics the hormonal fluctuations of the human menstrual cycle,[484] enabling the study of endometrial permeability to contraceptives and serving as a proof-of-concept for studying human embryo implantation, which is impossible to replicate using animal models. Ultrasonographic data has been used to build a 3D bioprinted endometrium for diagnosing congenital uterine anomalies.[485] Recently, the Human Endometrial Cell Atlas was published as a new reference for studying endometrial transcriptomics and guiding the development of human *in vitro* systems.[486]

Shifting resources away from inaccurate animal models and toward improvement in patient care would also profoundly affect outcomes. A recent study highlighted that misinterpreted symptoms are a major contributor to delayed endometriosis diagnoses.[487] To tackle this issue, the authors proposed a comprehensive approach that includes educating physicians, offering specialized courses for medical students, and integrating other healthcare professionals into the diagnostic and care processes.

The human menstrual cycle and endometrium are dynamic and unique to every individual, highlighting the need to prioritize personalized approaches using patient-derived models. Non-animal methods can revolutionize women's health research, offering more accurate models for disease study, drug testing, and precision medicine.

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Xenotransplantation

As the demand for organs grows, the once-experimental idea of using animals for transplants has evolved into a controversial push to breed pigs exclusively for organ harvesting, a practice known as xenotransplantation. There are multiple ways to improve our current system to increase access to viable human organs without xenotransplantation.

According to the United Network for Organ Sharing (UNOS), as of October 2024, over 104,000 people in the U.S. are waiting for organ transplants.[488] Despite this monumental and urgent need, the current system for managing, harvesting, and transporting human organs is highly inefficient. Human organs remain the most compatible and effective option for transplantation, yet inefficiencies in the system lead to the waste of many viable organs. Rather than resorting to genetically engineering, breeding, and killing pigs for organ harvesting, the focus should be on refining the Organ Procurement and Transplantation Network (OPTN), the current U.S. human organ donation system. Creating a separate xenotransplantation network would demand substantial government oversight and funding, adding complexity and potential inefficiency to an already challenging system. Instead, the most responsible and effective solution is to strengthen the current human organ donation process, ensuring patients receive the best possible transplant options.

Until recently, UNOS was the sole organization managing the OPTN in the U.S., but the organization has faced decades of criticism for poor management. A 2022 Senate Committee on Finance investigation revealed that organs procured by UNOS were often lost, damaged, delayed, or never collected.[489] A 2022 report by the National Academies of Sciences, Engineering, and Medicine concluded that the U.S. organ transplant system is inefficient, inequitable, inconsistent, and needs significant improvement.[490] Human organ transplantation is a critical and, by nature, scarce life-saving resource. Yet, one in five donor kidneys and one in ten donor livers were procured but never transplanted, primarily due to the systemic problems described above.[491]

Moreover, the current system often wastes already available organs. A study of kidney transplants from 2000 to 2015 found that in nearly 8,000 cases, one kidney was used while the donor's other kidney was discarded, often due to minor differences from ideal kidney organ donation criteria.[492] These discarded kidneys would likely function well, especially compared to long-term dialysis.[493] According to Dr Dalvin Roth, a Stanford professor and Nobel Prize recipient for his work on kidney exchange programs, transplant centres are pressured to reject kidneys because they are penalized for unsuccessful transplants.[494] However, transplant centres are not penalized for *rejecting* kidneys.[495] This system perpetuates the organ shortage as rejected kidneys may not meet an unrealistic threshold; considering the significant morbidity and mortality of long term dialysis, transplants offer far greater benefits to patients.[496] Reforming these criteria could significantly increase the number of available kidneys among other organs.

In response, President Biden signed the bipartisan *Securing the U.S. Organ Procurement and Transplantation Network Act* in 2023 to modernize the national transplant system.[497] This legislation aims to ensure patients receive high-quality human organs,[498] in contrast to animal organs, which harbour risks of rejection, zoonotic infections, and ethical concerns. In August 2024, the Health Resources and Services Administration announced that the OPTN Board of Directors, which governs national organ allocation policy, would be separately incorporated and independent from UNOS.[499] This is a critical step toward improving efficiency, but additional efforts to expand and improve the OPTN are needed as human organs remain the best option for transplant patients.

Xenotransplantation introduces additional risks, including transmitting pathogens from animals to humans, a phenomenon known as xenozoonosis. The FDA has recognized this as a significant risk, particularly for transplant patients who are inherently and medically immunosuppressed.[500] These infections could potentially spread to close contacts and the broader community, raising an ethical dilemma by pitting the duty to protect public health against the need to provide organ transplants for patients with end-stage organ failure.[501] Despite genetically engineering animals, raising them in pathogen-free facilities, and undergoing pathogen screening, viruses such as porcine cytomegalovirus or porcine roseolovirus are reported even after pre-transplant screening.[502] In May 2022, a pig heart transplant recipient died two months after his operation.[503] The autopsy revealed that the pig's heart carried undetected porcine cytomegalovirus and may have contributed to an unforeseen and untimely death in an immunocompromised individual.[504] As of July 2024, all xenotransplant recipients had died,[505] which may highlight the practice's futility but likely also reflects the fact that only high risk patients have been selected to receive this dangerous, experimental treatment. The risks of xenotransplantation are high compared to human organ transplants, which, when managed efficiently, remain the safest and most effective solution.

Rather than rely on xenotransplantation to solve the organ shortage, the U.S. should look towards systematic changes to increase the availability of human organs. For example, experts suggest adopting a "presumed consent" policy, recommended by a 2019 University of Michigan study.[506] In this system, organ donation is the "default" unless individuals opt out, a practice that has already increased donation rates in other countries.[507] Further, the U.S. can implement approaches similar to those of European countries that prioritize broad access to human organs and maximize the efficiency of their organ donation and transplantation systems.[508] Their success is driven by government commitment, an opt-out donation process, fostering a culture of trust and confidence in the system, and establishing dedicated institutions at multiple levels.[509] Further, proper hospital reimbursement ensures financial barriers do not impede participation.[510] These measures expand access to human organs and improve the efficiency of the transplantation system. By committing to improving the current U.S. organ donation system, policymakers can increase access to life-saving human organs without resorting to the ethically fraught, risky, and unnecessary practice of xenotransplantation.

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Toxicity Assessment

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

Please note that where tests are required for regulatory purposes, direct sources, such as the websites of the Organisation for Economic Co-operation and Development (OECD), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), and US Environmental Protection Agency (EPA), should be consulted for the most recent versions of test guidelines and guidance documents.

Approaches to Toxicity Assessment

Regulatory decision-making is facilitated by using all the relevant information available on a substance. One way to evaluate all the lines of evidence is to use an integrated approach to testing and assessment (IATA)[511] that considers multiple types of information to conclude the toxicity of a substance in a weight of evidence (WoE) approach. Information to consider includes existing data on the substance (e.g. from *in chemico*, *in vitro*, *in vivo* human, or *in vivo* animal studies), the physiochemical properties of the substance, data from non-testing approaches (e.g. QSARs and read-across), newly generated data (preferably from reliable and relevant non-animal methods), omics technologies (e.g. toxicogenomics), and use patterns or exposure scenarios. Data that are considered more reliable, relevant, and/or useful for the regulatory question have a greater influence on the final conclusion of the assessment. By assessing the available data together, it may be possible to conduct a robust risk assessment of the substance without generating new data through additional *in vivo* studies. (For example, see the Carcinogenicity section.) Additionally, a holistic data assessment will ensure that existing *in vivo* studies are not duplicated.

IATAs and WoE assessments often require expert judgement when integrating the results from combined approaches to make an informed conclusion for decision-making purposes. The methods, technologies, and frameworks that may be included in such approaches are accessible to those with the appropriate technological knowledge and there are various guidance documents and case studies to help in developing an IATA. For example, the OECD has published guidance on using defined approaches within an IATA.[512] Defined approaches consist of a fixed data interpretation procedure (e.g. a mathematical model or a rule-based approach) applied to data generated with a defined set of information sources to derive a prediction without the need for expert judgement.[513] For examples of defined approaches, see the Skin Sensitisation section.

Adverse outcome pathways (AOP) offer an additional framework for organising data collected from various methods and biological levels to assess the connections between key events and adverse effects. Unlike tests on animals, non-animal methods can reflect human-relevant biology and mechanisms of toxicity. AOPs comprise causally linked key events that connect chemical exposure to an adverse outcome. Non-animal tests that query specific key events in an AOP allow for a mechanistic understanding of whether an adverse outcome will occur following chemical exposure in humans. The OECD's AOP Development Programme supports the structured design of AOPs and provides guidance for using them within an IATA, as outlined in its Guidance

Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment. This initiative promotes the practical application of AOPs in regulatory settings.[514]

As mentioned above, consideration of exposure may also be part of an integrated approach. When human and environmental exposure to a substance is low, or when the physicochemical properties of a substance dictate that specific routes of exposure are not relevant, it may not be scientifically justified (or possible) to conduct toxicity tests for certain data requirements. When exposure is considered, the focus of regulatory decision-making can shift from a hazard-based "tick box" approach to a risk-centric approach that allows for the minimisation of tests on animals.[515]

However, a systematic framework is needed to evaluate individual methods' biological and toxicological relevance while also considering different exposure scenarios. Consolidating these approaches, the International Cooperation on Cosmetics Regulation (ICCR) has outlined key principles for integrating non-animal methods into a strategy for next generation risk assessment (NGRA).[516] NGRA is an exposure-led, hypothesis-driven risk assessment approach that integrates non-animal methods to ensure that chemical exposure does not cause harm.[517] The Partnership for the Assessment of Risks from Chemicals (PARC), an EU-funded initiative to modernise chemical safety assessments, also aims to make NGRA the default approach to chemical risk assessment in EU chemicals legislation.[518]

In addition to minimising animal testing, IATAs can leverage data and use high-throughput methods to assess a large number of chemicals more efficiently than tests on animals. They have the potential to fundamentally transform the current regulatory landscape by allowing more human-relevant decision-making based on both hazard and exposure assessments. Furthermore, with a concerted effort between relevant stakeholders, it is expected that similar gains are to be made with respect to integrated approaches for environmental protection.[519]

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[519]OECD. Guidance Document for the use of adverse outcome pathways in developing integrated approaches to testing and assessment (IATA). OECD Series on Testing and Assessment, No 260. Published 10 March 2017. Accessed 7 November 2024. https://doi.org/10.1787/44bb06c1-en.

Cosmetics

Legislation prohibiting either tests on animals for cosmetics purposes and/or the sale of cosmetics products containing ingredients tested on animals has been enacted in numerous regions, including the EU, the UK, India, Australia, Canada, Switzerland, South Korea, and Taiwan. In other countries, such as the US, legislation limiting the use of animal testing data has been brought in at a state level rather than on a national basis. This global shift away from tests on animals for cosmetics means that the sector has often been at the forefront of innovative safety assessment methods that have the potential to be applied more broadly.

However, despite the groundbreaking nature of these bans, certain regulatory requirements have undermined their full implementation. For example, companies can sell products in the EU and the UK even if they are tested on animals elsewhere, such as in China, provided the results of these tests are not used for meeting regulatory requirements under the relevant cosmetics regulations. Companies may pay for tests on animals required in other markets while using data from non-animal methods to meet EU or UK regulations. In the US, although there is no specific requirement to test cosmetics products or their ingredients on animals, in some instances, the US Food and Drug Administration (FDA) calls for such tests after products have been approved for market due to differing regional approaches to the classification of products.[520] Sunscreens, for example, are regulated as cosmetics in the EU but as over-the-counter pharmaceuticals in the US. The FDA has announced its intention to require that new tests on animals be conducted to keep sunscreens on the market if they contain any of the 12 specific active ingredients specified in a 2021 order. No similar request for new data on the same products has been requested in the EU or elsewhere.

Conflicts also exist in some areas between industrial chemicals and separate cosmetics legislation. The European Chemicals Agency (ECHA), backed by the European Commission, may mandates animal testing under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation to assess worker exposure and environmental risks for substances used exclusively in cosmetics products. For the assessment of substances used in cosmetics and other types of products, the REACH regulatory requirements apply to all human and environmental health endpoints regardless of worker exposure.[521] Similar legislative conflicts also exist in Australia and Canada.

As an array of non-animal methods and frameworks are now in use for the assessment of cosmetics, it is possible to eliminate the use of tests on animals while enhancing scientific rigour. For example, NGRA is a progressive approach involving hypothesis-driven, exposure-led evaluation combining *in silico*, *in chemico*, and *in vitro* methods to enable more accurate risk prediction and ensure the reliability of safety assessments.[522-525] NGRA frameworks can be adapted to make decisions on the safety of workers exposed to chemicals during product manufacture.[526] Likewise, OECD case studies demonstrate how tiered, flexible approaches to testing and assessment can be used to address safety concerns across different regulatory scenarios, from skin sensitisation to systemic and reproductive toxicity.[527] The Scientific Committee on Consumer Safety guidance on cosmetic safety assessments offers insights into how these innovative approaches can be applied effectively.[528] In addition, the International Collaboration on Cosmetics Safety, a coalition of cosmetics and personal care companies, ingredient manufacturers, trade associations, and NGOs,[529] is developing standardised best practice guidance on the use and understanding of new approach methodologies and NGRAs to further their regulatory acceptance.[530]

The mismatch between policy and scientific development for the assessment of cosmetics underscores the urgent need to take effective action to ensure that non-animal methods are used to protect consumers, workers, and the environment. In May 2023, the UK government took a significant step by halting the issuance of new licences for animal testing on ingredients used exclusively in cosmetics.[531] By November 2023, the Home Office confirmed that such tests had also ceased under all remaining legacy licences.[532] This move marks

the UK's progress towards completely ending animal testing for cosmetics. However, ingredients that are also used in other household products continue to undergo animal testing, as they are not fully exempt from testing requirements.

Full transparency is essential to foster informed consumer choices, ensure public trust, and address the erosion of legislation and policy designed to ensure that animals are not used to assess cosmetics products or their ingredients for all regulatory purposes.

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Ecotoxicity

Aquatic Toxicity and Bioaccumulation

Aquatic tests are conducted to measure the effects of chemicals on the environment and wildlife. In 2022, over 122,000 fish were used for regulatory use in the EU and Norway.[533] As assessment of bioaccumulation and aquatic toxicity is required in various regulatory frameworks, strategies to replace testing using aquatic animals are urgently needed.

A promising cytotoxicity assay using the RTgill-W1 cell line has been developed to assess[534] and the respective OECD test guideline was adopted in 2021.[535] This *in vitro* assay can potentially reduce or even replace the use of fish in the acute fish toxicity test.[536]

To enhance the prediction of acute fish toxicity, project 2.54 in the OECD Test Guidelines Programme work plan is developing a guidance document on IATAs for acute fish toxicity. This project is co-led by Austria and the International Council on Animal Protection in OECD Programmes (ICAPO), represented by PETA Science Consortium International.

Where testing on animals is still required, the number of animals used and the need to repeat studies can be reduced by careful application of OECD Guidance Document 23 on Aquatic Toxicity Testing of Difficult Substances and Mixtures.[537] This guidance document was updated in 2019 to provide information on approaches to aquatic toxicity testing of difficult-to-test chemicals. Particular attention was paid to updating the methods available for testing poorly water-soluble test chemicals while avoiding using solvents. Thus, the need for a solvent control group is eliminated, reducing the number of animals used for testing. In addition, the US and ICAPO (represented by PETA Science Consortium International) are co-leading project 2.55 in the OECD Test Guidelines Programme work plan on the use and analysis of control fish in toxicity studies. In this project, statistical analyses of existing data and simulations are being used to investigate whether it is possible to conduct aquatic toxicity studies using only one control when a solvent is used, further reducing the number of animals used.

Several non-animal methods are now available to reduce the number of fish used in bioaccumulation testing. In 2018, the OECD adopted two assays for the assessment of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes[538] and rainbow trout liver S9 subcellular fraction[539] and an associated guidance document.[540] 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 the 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 live fish in OECD Test No 305 on bioaccumulation in fish.[541]

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Avian Toxicity

Most regulatory authorities currently require avian toxicity tests to assess the potential ecological effects of chemicals on terrestrial birds. Three avian toxicity tests, including acute oral, dietary, and reproduction tests, are commonly required to fulfil regulatory requirements. In the acute oral and dietary tests, up to 120 birds are used. In the oral test, they are dosed with a chemical through gavage for one day, followed by a 14-day observation period. In the dietary test, they are fed the chemical for five days, followed by a three-day observation period. In the reproduction test, more than 120 adult birds are fed the chemical for eight to 10 weeks, and several hundreds to thousands of offspring are killed in order to examine potential adverse reproductive outcomes.

Scientists have raised concerns about the utility of avian tests to protect terrestrial species. The results of these tests, often conducted on two species, are used to extrapolate the potential effects on thousands of regional bird species. Additionally, food avoidance, regurgitation, and other issues caused by the methods used for dosing the birds have led to inaccurate toxicity estimates.

To address these concerns, PETA Science Consortium International collaborated with the US EPA to assess the use of avian oral and dietary tests in risk management decision-making.[542] The retrospective review examined 20 years of risk assessment data and found that the dietary test is generally not used for risk management. This study was used to support the EPA's 2020 policy entitled "Final Guidance for Waiving Sub-Acute Avian Dietary Tests for Pesticide Registration and Supporting Retrospective Analysis", which can prevent more than 700 birds from being subjected to toxicity tests each year and save resources that would be better spent developing fit-for-purpose non-animal methods for terrestrial toxicity testing.[543]

PETA Science Consortium International is undertaking a similar initiative to examine the use of two species in avian reproduction tests. This retrospective review will examine differences in avian species sensitivities to hundreds of pesticide active ingredients to analyse trends of how toxicity response are used in regulatory decision-making. The initiative aims to identify information that is not being used in regulatory decision-making. In addition to these projects, initiatives such as Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) aim to modernise ecological testing using predictive computational methods that have the potential to reduce testing on terrestrial animals while improving ecological protection.[544]

A lack of global alignment results in increased testing to meet unique regional requirements. For example, the European Commission and the Central Insecticides Board and Registration Committee (CIB&RC) in India require using a single test species for the avian reproduction test, yet the US EPA and Canada's Pest Management Regulatory Agency require two test species. Furthermore, the EPA allows waivers for the avian dietary test, and the dietary test is not required by the European Commission or in Japan, but it is still required by the CIB&RC and in China. Alignment is necessary to end globally the requirement for tests that have been shown not to provide useful information or that are affecting the quality of regulatory decision-making.

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[543]EPA OPP. Final guidance for waiving sub-acute avian dietary tests for pesticide registration and supporting retrospective analysis. Published February 2020. Accessed 25 August 2022. https://www.epa.gov/sites/default/files/2020-02/documents/final-waiver-guidance-avian-sub-acute-dietary.pdf.

[544]LaLone CA, Villeneuve DL, Lyons D, et al. Editor's highlight: Sequence alignment to predict across species susceptibility (SeqAPASS): A web-based tool for addressing the challenges of cross-species extrapolation of chemical toxicity. *Toxicol Sci*. 2016;153(2):228-245.

Endocrine Disruption

Endocrine disruptors are natural or synthetic chemicals that interfere with the body's endocrine system,[545] influencing various responses in biological pathways responsible for regulating fundamental biological functions, such as growth, development, reproduction, energy balance, metabolism, or body weight regulation. From a regulatory chemical safety perspective, the most investigated endocrine pathways are the oestrogen, androgen, thyroid, and steroidogenesis (EATS) systems and, to a lesser degree, non-EATS systems, such as the retinoid pathway.[546]

Much is understood about the complex mechanisms through which chemicals can interfere with endocrine pathways in humans[547] and wildlife.[548] Numerous AOPs related to endocrine disruption are included in the AOP Wiki,[549] and the OECD has published several case studies on IATAs.[550] *In vivo* tests assessing endocrine disruption demonstrate high variability (e.g. stress experienced by the animal can significantly influence the study's outcome) and low sensitivity, and they are unlikely to detect relevant endocrine disrupting events.[551] Classical endpoint studies are not appropriate in this area and need to be replaced by *in vitro* studies in which the multiple factors that could affect test results can be more effectively controlled.

From 2019 to 2024, eight projects under the European Cluster to Improve Identification of Endocrine Disruptors (EURION), with €50 million of funding from the European Commission, focused on the development of tools aiming to improve regulatory assessment of endocrine-related effects (thyroid hormone system disruption, metabolic disorders, developmental neurotoxicity, and female fertility) and reduce the reliance on animal testing. A concluding policy brief for the EURION project concluded that support is needed for faster implementation of scientific findings into test methods as well as for the update of test requirements in chemical regulations to include newly developed tests.[552]

The US EPA's Office of Research and Development (ORD) is developing *in silico* and *in vitro* assays as well as AOPs to support the robust assessment of chemicals for effects on the endocrine system. For example, the EPA's Toxicity Forecaster (ToxCast) ranks and prioritises chemicals using more than 700 high-throughput screening assays and computational toxicology approaches, which cover a variety of relevant cellular responses and signalling pathways.

Following a comparative study of ToxCast oestrogen pathway assay results and uterotrophic assay results,[553] the EPA announced that it will accept the data from the ToxCast ER Bioactivity Model as an alternative to at least one animal test[554,555] – the uterotrophic assay – that screens for effects on the oestrogen pathway.[556] In the EU, the ToxCast ER Bioactivity Model is currently accepted as a source of *in vitro* mechanistic mode of action information required as part of the identification of substances as endocrine disruptors under the current regulatory framework for biocides and plant protection products.

In collaboration with other organisations, the EU Joint Research Centre and the EPA ORD are developing and assessing the validity of sets of relevant assays based on the thyroid AOP.[557] In 2024, the OECD added two of these assays, targeting different molecular initiating events related to thyroid pathway disruption, to their workplan for test guideline development.

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[546]La Merrill MA, Vandenberg LN, Smith MT, et al. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nat Rev Endocrinol*. 2020;16(1):45-57.

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[548]Metcalfe CD, Bayen S, Desrosiers M, et al. An introduction to the sources, fate, occurrence and effects of endocrine disrupting chemicals released into the environment. *Environ Res*. 2022;207:112658.

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Eye Irritation/Corrosion

To assess eye irritation and corrosion using the Draize 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 in 1944, and advanced replacement methods have since been developed and shown to be as or more reliable and relevant than the rabbit test. For example, an analysis of 491 chemicals with at least two rabbit eye tests showed that the probability of obtaining the same UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification more than once was 73% for GHS category 1 (causes serious eye damage), 32.9% for GHS category 2A (irritant), 15.5% for GHS category 2B (mild irritant), and 93.9% for no category (non-irritating).[558] Importantly, these results showed that there was a 10.4% chance that a chemical once identified as causing serious irreversible damage (category 1) would later be identified as non-irritating (no category).

Robust and defined non-animal methods are available to fully replace the Draize test without the need for expert judgement or a WoE assessment:

- **OECD Test No 492B: Reconstructed Human Cornea-Like Epithelium (RhCE) Test Method for Eye Hazard Identification**, which may be used to identify chemicals not requiring classification (GHS no category) and

those requiring eye irritation classification (GHS category 2) and serious eye damage classification (GHS category 1).

- **OECD Test No 467: Defined Approaches for Serious Eye Damage and Eye Irritation.** The defined approaches in OECD Test No 467 are based on the following:
  a) Physicochemical properties and *in vitro* data from **OECD Test No 492: Reconstructed Human Cornea-Like Epithelium (RhCE) Test Method** and **OECD Test No 437: Bovine Corneal Opacity and Permeability (BCOP) Test Method** for neat non-surfactant liquids
  b) *In vitro* data from **OECD Test No 491: Short Time Exposure (STE) In Vitro Test Method** and **OECD Test No 437** for neat and/or diluted non-surfactant liquids or solids dissolved in water
  c) *In vitro* data from **OECD Test No 437** and **OECD Test No 492** for neat solids. The defined approaches may be used to identify chemicals not requiring classification (GHS no category) and those requiring eye irritation classification (GHS category 2) and serious eye damage classification (GHS category 1).

Other *in vitro* methods may be combined – as outlined in the OECD guidance document on an IATA of serious eye damage and irritation[559] – to fully replace the Draize test:

- **OECD Test No 494: Vitrigel-Eye Irritancy Test Method** – This test may be used to identify chemicals not classified for eye irritation or causing serious eye damage (GHS no category).
- **OECD Test No 496: *In Vitro* Macromolecular Test Method** – This test may be used to identify chemicals causing serious eye damage (GHS category 1) and/or not requiring classification.
- **OECD Test No 460: Fluorescein Leakage Test Method** – This test may be used to identify chemicals causing serious eye damage (GHS category 1). It is recommended as an initial step within a top-down approach to identifying ocular corrosives or severe irritants.
- **OECD Test No 438: Isolated Chicken Eye Test Method** – This test may be used to identify chemicals causing serious eye damage (GHS category 1) or not requiring classification. It is 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. Certain methods will be more appropriate than others, depending on the applicability domain of the method, purpose of testing, and type of test chemical (e.g. surfactants or solids).

The EPA currently accepts the use of *in vitro* and *ex vivo* methods for determining eye irritation and corrosion when classifying industrial chemicals, antimicrobial cleaning products, and, on a case-by-case basis, other pesticide products. The EPA Office of Pollution Prevention and Toxics published a decision framework in 2024 which discourages prospective Draize tests for new chemical products,[560] and in 2015, the Office of Pesticides Programs (OPP) published a guidance document describing the testing framework that industry can use for this endpoint.[561] OPP also published on its webpage[562] a paper in which the authors proposed defined approaches combining *in vitro* and *ex vivo* methods to assess eye irritation/corrosion potential of agrochemical formulations.[563] The paper was co-authored with PETA Science Consortium International, the US National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and others.

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Genotoxicity and Carcinogenicity

Genotoxicity

The major genotoxicity endpoints to be evaluated for regulatory purposes are gene mutation, structural chromosomal aberrations (clastogenicity), and numerical chromosomal aberrations (aneuploidy). OECD test guidelines for assessing genotoxicity *in vitro* cover one or two endpoints simultaneously:

- **OECD Test No 471: Bacterial Reverse Mutation Test** – This test, commonly known as the Ames test, uses amino acid–requiring *Salmonella typhimurium* and *Escherichia coli* to detect point mutations by base substitutions or frameshifts.
- **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. This assay detects structural and numerical chromosomal aberrations.
- **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 473: *In Vitro* Mammalian Chromosomal Aberration Test** – This test identifies chemical substances that cause structural chromosomal aberrations.
- **OECD Test No 476: *In Vitro* Mammalian Cell Gene Mutation Test Using the *Hrpt* and *xrpt* Genes** – These tests can detect gene mutations induced by chemicals.

The assessment of genotoxicity for regulatory purposes typically follows a step-wise approach starting with a core battery of *in vitro* tests (e.g. the Ames test, micronucleus test, and chromosome aberration test). The need to follow up *in vitro* tests with *in vivo* tests depends on the results and regulatory requirements. For example, in the case of the EU's industrial chemicals and biocides regulations, a positive result in any of the required *in vitro* tests must be followed up with an *in vivo* test.[564,565] However, if a substance produces negative results in the *in vitro* tests, it can be categorised as having no genotoxic potential and no further genotoxicity testing is required. Conversely, for some chemical classes, *in vivo* testing is required regardless of the *in vitro* test results (e.g. plant protection products and pharmaceuticals).[566,567]

Appropriate data from *in silico* studies (e.g. QSARs and read-across) can help reduce the requirement to conduct *in vivo* tests. The EURL ECVAM–consolidated genotoxicity and carcinogenicity database published in the EURL ECVAM collection of the Joint Research Centre (JRC) data catalogue, for example, provides substantial resources for read-across.[568]

Furthermore, advanced *in vitro* methods can provide follow-up and de-risking options for use in a WoE approach. For example, the *in vitro* transcriptomic biomarker responsive to DNA-damage-inducing (DDI) agents, TGx-DDI,[569,570] and the ToxTracker assay[571-573] can provide information on the mode of action of potential genotoxicants and have been submitted to formal regulatory "qualification" programmes.[574,575] Data generated using the ToxTracker assay and read-across have been used in the EU's REACH dossiers.[576]

The three-dimensional reconstructed skin micronucleus and comet assays are additional non-animal methods that can be used to follow up positive results from standard *in vitro* genotoxicity assays for dermally applied compounds. They present an important opportunity to avoid the use of animals for genotoxicity testing.[577,578] The information requirements for genotoxicity assessment of cosmetics[579] may already invoke the micronucleus test using three-dimensional reconstructed human skin or a comet test using either mammalian cells or three-dimensional reconstructed human skin. Rapid progress in the development of three-dimensional liver and airway models holds the prospect of assessing the genotoxic potential of compounds administered by the oral or inhalation route in the near future without using animals.[580]

Non-animal methods are gaining ground internationally. Generating comprehensive data based on these methods and developing case studies, such as that on coumarin used in cosmetics products, is an important component of supporting the adoption of next generation risk assessment.[581,582]

The genotoxicity[583] and mutagenicity[584] case studies on IATA, under the OECD IATA case studies project,[585] illustrate feasible approaches to developing adequate safety assessment guidelines for systemic genotoxicity risk assessment without animal testing.

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[565]ECHA. Guidance on the Biocidal Products Regulation. Volume III: Human health, Part A: Information requirements. Version 1.2. Published May 2018. doi:10.2823/443383.

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Carcinogenicity

The assessment of carcinogenicity often requires testing on rats and/or mice for the majority of their lives (up to two years). The test requires a minimum of 400 rats and/or mice per chemical assessment (OECD Test No 451, No 452, and No 453).

While carcinogenicity studies in animals are still routinely conducted, the test has been under scientific scrutiny since the early 1970s for its lack of reproducibility[586] and its inability to predict human outcomes.[587] Namely, there are two flawed assumptions that underlie these bioassays: (1) rodent carcinogens are human carcinogens, and (2) high-dose chemical exposure in rodents is indicative of an environmentally relevant dose. Both have been proved incorrect by 50 years' worth of carcinogenicity data. Decades of scientific reviews highlight the overall lack of reliability in the rodent cancer bioassays to predict human cancers.[588-593]

For example, in an assessment of 202 pesticide evaluations from a review programme conducted by the EU, 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.[594] 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.[595] This study was used to support an international collaboration that developed a WoE approach to fulfil some of the carcinogenicity test requirements without the two-year test on rats.[596,597] The collaboration resulted in an addendum to the guideline for carcinogenicity assessment of pharmaceuticals (ICH S1B) – thus providing an opportunity to spare 400 animals per pharmaceutical regulatory evaluation.[598] A similar effort called Rethinking chronic toxicity and Carcinogenicity Assessment for Agrochemicals Project (ReCAAP), led by PETA Science Consortium International, developed a framework to support a WoE-based assessment of agrochemicals without long-term carcinogenicity testing on rats and mice.[599] The ReCAAP framework has since been accepted for publication by the OECD Working Party

for Hazard Assessment (WPHA) whereby eight global regulatory bodies endorsed the WoE-based approach to fulfilling safety assessment needs – without conducting the lifetime tests on rats and mice.[600]

Additional activities are ongoing to develop a framework to provide a modular strategy for assessing carcinogenicity in non-genotoxic chemicals, including efforts from the OECD Working Party for the Test Guideline Program (WNT) expert group on non-genotoxic carcinogens (NGTxC). This framework offers a modular approach to evaluating and integrating in vitro and in silico data into an AOP-style for assessing bioactivity that could potentially lead to carcinogenicity.[601]

The OECD WNT is also assessing the *in vitro* cell transformation assays (CTA) for their ability to recapitulate a multistage process that models some aspects of *in vivo* carcinogenesis. The CTA has the potential to detect both genotoxic and non-genotoxic carcinogens.[602] 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.[603] Following a study in which the Bhas 42 CTA was tested with 98 substances – including known human carcinogens – the OECD has recommended this assay be used as part of a testing strategy to help assess potentially cancer-causing substances.[604,605] 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 *in vivo* testing.[606,607]

Several computational tools and models further help to assess carcinogenicity potential. Structural alerts flagging potential non-genotoxic carcinogens have been incorporated into the OECD QSAR Toolbox.[608] Additionally, the EPA has published a computer model, OncoLogic™, to evaluate chemicals for carcinogenic potential,[609] and commercial options are also available, such as those from Lhasa Limited, MultiCASE, UL Cheminformatics, and Instem. Ultimately, identifying DNA-reactive chemicals with the Ames test or genotoxic structural alerts can potentially be combined with identifying non-genotoxic carcinogens using structural alerts, leaving CTAs to model most of what is left unexplained in a WoE approach.

Given the complexity of carcinogenesis, experts recognise that there needs to be an integration of new approaches (e.g. *in silico* or *in vitro*) to support a fit-for-purpose WoE-based safety assessment.[610] Fortunately, initiatives are underway to facilitate the integration of methods to ultimately achieve an animal-free, rapid, and human-relevant carcinogenicity assessment for chemical and pharmaceutical regulation.[611-614]

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Phototoxicity

Substances that absorb light in the UV and visible range (290 to 700 nm) and can reach the skin or eyes may require testing for potential phototoxicity. Phototoxicity is the toxic response to a topically or systemically administered substance that occurs after exposure to light. It can cause symptoms ranging from first-degree burns (redness, itching, and pain) to full-thickness third-degree burns. Phototoxicity, often also called photosensitivity, is a well-known adverse effect of many drugs, including antimicrobials, nonsteroidal anti-inflammatory drugs, diuretics, and chemotherapeutic agents.[615]

Phototoxicity testing for systemically or topically administered compounds has been conducted in various species, including guinea pigs, mice, and rats. However, no validated or standardized *in vivo* study design has been established.[616,617] By contrast, so far, three OECD test guidelines have been developed using *in chemico* and *in vitro* methods to assess phototoxicity:

- **OECD Test No 495: Ros (Reactive Oxygen Species) Assay for Photoreactivity** – This *in chemico* method measures the ability of a substance to create reactive oxygen species under exposure to artificial sunlight.
- **OECD Test No 432: *In Vitro* 3T3 NRU Phototoxicity Test** – This test measures the viability of a mouse cell line incubated with a potential phototoxicant and exposed to light.
- **OECD Test No 498: *In Vitro* Phototoxicity – Reconstructed Human Epidermis Phototoxicity Test Method** – In this test, a three-dimensional reconstructed human epidermis model is incubated with the potential phototoxicant and exposed to light.

OECD Test No 498 is based on a similar principle as **OECD Test No 432** but uses a three-dimensional reconstructed human skin model instead of the mouse cell line. This expands the applicability domain to a wider selection of substances, including final formulations, complex mixtures, or dermatological patches.[618] Substances with an extreme pH can also be tested using the three-dimensional skin models.

These OECD test guidelines can be combined with other physico-chemical assessments and *in vitro* and *in silico* approaches – as outlined in the OECD's Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Phototoxicity Testing – without animal testing to assess a substance's phototoxic potential.[619]

[615]Pharmacy Times. Drug-induced photosensitivity: Focus on antibiotics. *Pharmacy Times*. Published 24 August 2016. Accessed 25 August 2022. https://www.pharmacytimes.com/view/drug-induced-photosensitivity-focus-on-antibiotics.

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Pyrogenicity

Regulators require testing to demonstrate that specific drugs and medical devices are not contaminated with substances that trigger a fever response. These substances, collectively termed pyrogens, are chemically and structurally diverse but generally prompt 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. Two *in vitro* methods are available that detect pyrogens:

- **Monocyte activation test (MAT)**, defined in *European Pharmacopoeia* (*Ph Eur*) general chapter 2.6.30 and permitted in *United States Pharmacopeia* (*USP*) general chapter 151
- **Recombinant Factor C (rFC) assay**, defined in *Ph Eur* general chapter 2.6.32 and, beginning May 2025, in *USP* general chapter 86

Even though the human fever response mechanism is well understood, most global regulators still commonly require two animal-based tests to assess pyrogen contamination. In the rabbit pyrogen test (RPT), rabbits are injected with a test substance and subsequently restrained for three hours, during which changes in their body temperature are monitored rectally. In the EU and Norway alone, more than 125,000 rabbits were used between 2018 and 2022 in the RPT.[620] Although some countries appear to have ceased using the RPT, others like France and Spain still used over 6,000 animals each in 2022 – 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.[621]

The limulus amoebocyte lysate test (LAL), requires the use of haemolymph from captured horseshoe crabs and detects only bacterial endotoxins and no other pyrogens. After the bleeding process, up to 30% of the crabs die. Those who recover are less likely to survive in nature.[622] A synthetic version of the LAL, in which the haemolymph is replaced by a recombinant reagent (the rFC assay), is available to test for bacterial endotoxins. The rFC assay is a reliable and animal-friendly test with equal or superior performance to LAL.[623]

Since 2010, the *in vitro* monocyte activation test (MAT), capable of detecting both endotoxin and non-endotoxin pyrogens, has been validated and included in the *Ph Eur*.[624] In the MAT, drugs and medical devices are incubated with human whole blood or isolated human monocytes. After this exposure period, tests measure pro-inflammatory cytokines released by monocytes.[625] 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.[626]

Regulators in the EU, India, the UK, and the US – as well as the pharmacopoeias used in these regions – all allow the use of the MAT and rFC following product-specific validation. Nevertheless, tests on animals are still used despite their well-documented limitations.[627] To eliminate the use of animals in pyrogen tests, regulatory authorities and standards organisations must make an increased effort to integrate and harmonise a preference for non-animal tests in international testing requirements and to encourage drug and device manufacturers to use and submit data from these tests in their product dossiers. In September 2018, participants at a workshop organised by PETA Science Consortium International and NICEATM discussed non-animal approaches to medical device pyrogen testing and called for more opportunities for training and education to increase the use of the MAT for regulatory purposes.[628]

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 usability of the method and to emphasise that it is considered a replacement for pyrogen tests using animals.[629,630] This endorsement is repeated in statements from the European Medicines Agency,[631] and the *Ph Eur* Commission has announced that sanction of the RPT will be officially removed from the *Ph Eur* in 2025.[632] 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.[633] In the 8th edition of *Indian Pharmacopoeia*, the Indian Pharmacopeia Commission revised the pyrogen testing general chapter, introduced the monograph on the MAT, and replaced the RPT with LAL.[634] However, due to unclear guidance and regulatory ambiguity about the applicability of the MAT as a stand-alone pyrogen test, the RPT and LAL continue to be used.

[620]European Commission. Summary Report on the statistics on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2022. Table 20: Number of uses for pyrogenicity testing per country between 2018 and 2022. Accessed 18. September 2024. https://circabc.europa.eu/ui/group/8ee3c69a-bccb-4f22-89ca-277e35de7c63/library/051e5787-7746-46cf-8a0d-310f84fd1900/details?download=true.

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[630]EDQM. European Pharmacopoeia Commission adopts revised general chapter on monocyte-activation test to facilitate reduction in testing on laboratory animals. 23 June 2016. Accessed 17 January 2025. https://www.edqm.eu/en/d/183809.

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Reproductive and Developmental Toxicity

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

Developmental toxicity studies for chemical and pharmaceutical human safety assessment are primarily performed using rats. However, many regulatory frameworks – including the biocidal products and plant protection product regulations and, in some circumstances, REACH in the EU – require registrants to submit test results using a second species, usually rabbits, under the assumption of interspecies differences in

sensitivity to developmental effects. These studies use a large number of animals. For example, a study estimated the total number of animals used for reproductive and developmental endpoints in existing registration dossiers from the public ECHA database (as of December 2022) to be approximately 2.7 million.[635]

None of the *in vivo* methods used for testing reproductive and developmental toxicity have been formally validated for their relevance to humans,[636] and retrospective evaluations demonstrate their significant limitations and the subjectivity of data interpretation.[637,638] Therefore, significant investment is required to develop human-relevant non-animal methods. Recently, 42 AOPs from the AOP-wiki, relevant for mammalian reproductive toxicity, were included in an AOP network for oestrogen-, androgen- and steroidogenesis-mediated reproductive toxicity, covering effects on hormone levels or hormone activity, cancer outcomes, male and female reproductive systems, and overall effects on fertility and reproduction.[639]

Due to the extensive knowledge about key events of reproductive and developmental toxicity, many promising assays and test batteries have been developed. The EU ReProTect project, which aimed to develop innovative methods of assessing reproductive toxicity, demonstrated that a battery of several *in vitro* and *in silico* tests, including the embryonic stem cell test, could be used to provide valuable information on adverse effects during embryonic development.[640] A novel human stem cell–based biomarker assay, ReproTracker®, identifies the teratogenicity potential of chemicals.[641] Additionally, a battery of diverse assays was developed, including the CALUX transcriptional activation assay (for steroidogenic activity), ReProGlo assay (for body axis patterning and cell fate specification), embryonic stem cell test (for differentiation into cardiomyocytes), and zebrafish embryotoxicity assay.[642]

In addition, the EU-ToxRisk project integrates advancements in cell biology, “omic” technology, systems biology, and computational modelling to define the complex chains of events that link chemical exposure to toxic outcomes. The project focuses on repeat-dose systemic toxicity and developmental and reproductive toxicity. 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.[643] The OECD, JRC, European Food Safety Authority (EFSA), and the EPA have developed recommendations to demonstrate how the integration of a battery of *in vitro* assays can be used to determine the potential of chemical developmental neurotoxicity, and the partner agencies are working on case studies that apply to different chemical classes.[644-646] A study compared *in vitro* bioactivity-based points of departure ($POD_{Bioactivity}$) with points of departure from oral repeat-dose, developmental, and reproductive studies ($POD_{Traditional}$) used in risk assessment. For 43 out of 46 of the examined chemicals, $POD_{Bioactivity}$ was more conservative than the lowest $POD_{Traditional}$, demonstrating confidence in using *in vitro* bioactivity as a surrogate lower bound estimate of *in vivo* adverse effect levels – a strong indication that using $POD_{Bioactivity}$ would be equally or more protective than using $POD_{Traditional}$.[647]

While the field is gradually moving towards a range of integrative strategies in order to cover the majority of possible mechanisms, much more research is required.

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[636]Rovida C, Longo F, Rabbit RR. How are reproductive toxicity and developmental toxicity addressed in REACH dossiers? *ALTEX*. 2011;28(4):273-294.

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[639]Zilliacus J, Draskau MK, Johansson HKL, Svingen T, Beronius A. Building an adverse outcome pathway network for estrogen-, androgen- and steroidogenesis-mediated reproductive toxicity. *Front Toxicol*. 2024;6:1357717.

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[642]van der Burg B, Pieterse B, Buist H, et al. A high throughput screening system for predicting chemically-induced reproductive organ deformities. *Reprod Toxicol*. 2015;55:95-103.

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[645]OECD. Initial recommendations on evaluation of data from the developmental neurotoxicity (DNT) *in-vitro* testing battery. OECD Series on Testing and Assessment, No 377. Published 3 November 2023. Accessed 10 December 2024. https://doi.org/10.1787/91964ef3-en.

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[647]*Ibid*.

Skin Irritation/Corrosion

Skin irritation and corrosion tests for chemicals are required or recommended by several regulatory agencies. In the animal test, a test substance is applied to the shaved skin of a rabbit, 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, scabs, and scarring.

Skin irritation studies using animals have been used for years, even though they have been shown to be generally poor predictors of human skin reactions and highly variable.[648] For example, a comparison of data from rabbit tests and four-hour human skin patch tests for 65 substances found that 45% of classifications of chemical irritation potential based on tests on animals were incorrect.[649]

There are opportunities to avoid the animal test based on criteria described in OECD guidance document no 237.[650] Furthermore, the OECD has developed an IATA for skin irritation using *in vitro* skin irritation and corrosion methods that avoid or minimise animal use.[651]

- **OECD Test No 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis (RHE) Test Method** – This test may be used for the hazard identification of irritant chemicals (substances and mixtures), in accordance with the GHS, as category 2, or unclassified chemicals. It may be used as a stand-alone test or in a tiered testing strategy.
- **OECD Test No 431: *In Vitro* Skin Corrosion: RHE Test Method** – This test may be used to identify corrosive chemical substances and mixtures. It may also distinguish between severe and less severe skin corrosives.
- **OECD Test No 435: *In Vitro* Membrane Barrier Test Method for Skin Corrosion** – This test allows corrosive chemicals to be categorised as one of the three GHS corrosivity subcategories.

Recently, **OECD Test Guideline No. 439** was validated for use in assessing the ability of medical device extracts to cause skin irritation, and the ISO 10993 guidance has been updated to include this test.[652]

[648]Rooney JP, Choksi NY, Ceger P, et al. Analysis of variability in the rabbit skin irritation assay. *Regul Toxicol Pharmacol.* 2021;122:104920.

[649]Robinson MK, Cohen C, de Fraissinette AB, Ponec M, Whittle E, Fentem JH. Non-animal testing strategies for assessment of the skin corrosion and skin irritation potential of ingredients and finished products. *Food Chem Toxicol.* 2002;40(5):573-592.

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[651]OECD. Guidance document on an integrated approach on testing and assessment (IATA) for skin corrosion and irritation. OECD Series on Testing and Assessment, No 203. Published 13 April 2017. Accessed 17 January 2025. https://doi.org/10.1787/9789264274693-en.

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Skin Sensitisation

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 injecting a test substance into – or applying a test substance to the shaved skin of – guinea pigs in the guinea pig maximisation test or applying it to the ears of mice in the local lymph node assay.

The regulatory requirement to test for skin sensitisation can be met with a defined approach, as described in **OECD Test No 497: Defined Approaches on Skin Sensitisation**, using a combination of *in chemico* and *in vitro* assays that each addresses a different key event in the AOP.[653] The "2 out of 3" defined approach provides sufficient information for hazard identification, and the integrated testing strategies (ITSv1 and ITSv2) collate information from two of the *in vitro* assays included in the guidelines listed below, along with *in silico* predictions, to predict hazard and potency.

- **OECD Test No 442C: Key Event-Based Test Guideline for *In Chemico* Skin Sensitisation Assays Addressing the Adverse Outcome Pathway Key Event on Covalent Binding to Proteins** – This test 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 addresses the second key event of the skin sensitisation AOP.
- **OECD Test No 442E: *In Vitro* Skin Sensitisation Assays Addressing Key Event on Activation of Dendritic Cells** – This test addresses the third key event of the skin sensitisation AOP.

When compared to human data, the non-animal approaches to predicting skin sensitisation are as good as or better than the local lymph node assay.[654]

[653]OECD. Guideline No 497: Defined approaches on skin sensitisation. OECD Guidelines for the Testing of Chemicals, Section 4. Published 4 July 2023. Accessed 10 December 2024. https://doi.org/10.1787/b92879a4-en.

[654]Hoffmann S, Kleinstreuer N, Alépée N, et al. Non-animal methods to predict skin sensitization (I): The Cosmetics Europe database. *Crit Rev Toxicol.* 2018;48(5):344-358.

Systemic Toxicity

Acute Systemic Toxicity

To determine the danger of exposure to a product or chemical, a substance is administered to animals through the oral, dermal, or inhalation routes. Acute toxicity refers to adverse effects observed following one high level of exposure to a substance for a short duration (up to 24 hours). In these tests, 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 determined. The LD_{50} test and its adaptations have never been scientifically validated, and their accuracy in predicting chemical effects in humans remains in question. An analysis of the variability of the acute oral toxicity animal test showed that there is 78% or 74% accuracy in obtaining the same EPA or GHS classification, respectively, if the same chemical is tested more than once,[655] while another analysis of existing acute oral LD_{50} data demonstrated that replicate studies result in the same hazard categorisation on average 60% of the time.[656] This second study demonstrated that inherent biological or protocol variability most likely underlies the variance in the results.

When scientific justification is provided, regulatory authorities may allow acute toxicity assessment without testing on animals. The OECD has published guidance for waiving or bridging acute toxicity testing,[657] and the EPA has published similar guidance for pesticides and pesticide products.[658] This includes the use of existing data for read-across and the consideration of the physicochemical properties of the test substance.[659,660]

[655]Kleinstreuer NC, Karmaus AL, Mansouri K, Allen DG, Fitzpatrick JM, Patlewiczc G. Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation. *Comput Toxicol*. 2018;8:21-24.

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[657]OECD. Guidance document on considerations for waiving or bridging of mammalian acute toxicity tests. OECD Series on Testing and Assessment, No 237. Published 13 April 2017. Accessed 17 January 2025. https://doi.org/10.1787/9789264274754-en.

[658]EPA OPP. Guidance for waiving or bridging of mammalian acute toxicity tests for pesticides and pesticide products (acute oral, acute dermal, acute inhalation, primary eye, primary dermal, and dermal sensitization). Published 1 March 2012. Accessed 17 January 2025. https://www.epa.gov/sites/default/files/documents/acute-data-waiver-guidance.pdf.

[659]Strickland J, Haugabrooks E, Allen DG, et al. International regulatory uses of acute systemic toxicity data and integration of new approach methodologies. *Crit Rev Toxicol*. 2023;53(7):385-411.

[660]Borba JVB, Alves VM, Braga RC, et al. STopTox: An *in silico* alternative to animal testing for acute systemic and topical toxicity. *Environ Health Perspect*. 2022;130(2):27012.

Repeat-Dose Systemic Toxicity

In repeat-dose toxicity studies, animals are exposed repeatedly to substances for up to one month (sub-acute), up to three months (sub-chronic), or up to several years (chronic) in order to measure the effects of multiple chemical exposures. Chemicals are usually administered to animals using oral gavage unless humans are more likely to be exposed via another route. Like other endpoints, there is evidence that regulatory studies using animals to assess repeat-dose toxicity are not fit for purpose, and there is a clear need to develop new approaches. In 2020, Pham and colleagues evaluated the sources of variability in the values used to derive safe exposure levels from a variety of repeat-dose studies in rodents and found that approximately one-third of the total variance could not be accounted for through considerations of study differences, e.g. administration route or study type.[661,662]

The assessment of repeat-dose toxicity is a standard requirement in human safety evaluation, and while read-across approaches are accepted for regulatory purposes, other non-animal methods have yet to gain full acceptance. To address this gap in the use of non-animal methods, various projects across academia, industry,

and regulatory bodies have proposed diverse sets of high-content, high-throughput, and "omic" technology-based *in vitro* and *in silico* assays. These initiatives focus on developing non-animal testing methods to derive *in vitro* points of departure, predict maximal plasma concentrations, or calculate bioactivity exposure ratios.[663-666] An OECD case study on the use of an IATA for systemic toxicity demonstrates the application of such advanced methodologies.[667]

While the development and regulatory implementation of *in vitro* testing systems advances, the number of animals used for repeat-dose toxicity testing under various regulatory frameworks may be immediately reduced by the extrapolation of points of departure from sub-chronic to chronic studies.[668] A review of points of departure (NOAELs or LOAELs) determined from *in vivo* studies with food additives showed that the chronic values may be extrapolated with high confidence from sub-chronic studies, supporting previous analyses of other types of substances, including industrial chemicals and pesticides. The risk assessment and derivation of health-based guidance values may be further strengthened by a precautionary application of an additional uncertainty factor of 2 to account for any outlying values – an approach recommended by EFSA and supported by data from a number of recent studies.[669]

[661]Pham LL, Watford SM, Pradeep P, et al. Variability in *in vivo* studies: Defining the upper limit of performance for predictions of systemic effect levels. *Comput Toxicol*. 2020;15:100126.

[662]Guth S, Roth A, Engeli B, et al. Comparison of points of departure between subchronic and chronic toxicity studies on food additives, food contaminants and natural food constituents. *Food Chem Toxicol*. 2020;146:111784.

[663]Zobl W, Bitsch A, Blum J, et al. Protectiveness of NAM-based hazard assessment – which testing scope is required? *ALTEX*. 2024;41(2):302-319.

[664]Middleton AM, Reynolds J, Cable S, et al. Are non-animal systemic safety assessments protective? A toolbox and workflow. *Toxicol Sci*. 2022;189(1):124-147.

[665]Reardon AJF, Farmahin R, Williams A, et al. From vision toward best practices: Evaluating *in vitro* transcriptomic points of departure for application in risk assessment using a uniform workflow. *Front Toxicol*. 2023;5:1194895.

[666]Hatherell S, Baltazar MT, Reynolds J, et al. Identifying and characterizing stress pathways of concern for consumer safety in next-generation risk assessment. *Toxicol Sci*. 2020;176(1):11-33.

[667]OECD. Case study on use of an integrated approach for testing and assessment (IATA) for systemic toxicity of phenoxyethanol when included at 1% in a body lotion. OECD Series on Testing and Assessment, No 349. Published 27 October 2021. Accessed 10 December 2024. https://one.oecd.org/document/ENV/CBC/MONO(2021)35/En/pdf.

[668]Guth S, Roth A, Engeli B, et al. Comparison of points of departure between subchronic and chronic toxicity studies on food additives, food contaminants and natural food constituents. *Food Chem Toxicol*. 2020;146:111784.

[669]EFSA Scientific Committee. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA J*. 2012;10(3):1-32.

Oral Route

NICEATM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) carried out a project to develop predictive models for acute oral systemic toxicity.[670] The outcome was the Collaborative Acute Toxicity Modelling Suite (CATMoS) tool for predicting acute oral toxicity to meet various regulatory needs, which were presented at an April 2018 workshop.[671] It generated 139 predictive models using data from about 12,000 chemicals. A consensus model was built, combining the individual models after weighting their individual performance. CATMoS is implemented through Open Structure-Activity/Property Relationship App (OPERA), a freely available and open-source QSAR tool.[672] This model is routinely optimised and further evaluated,[673] and updates are available on the NICEATM Integrated Chemical Environment (ICE) and EPA websites.[674] PETA Science Consortium International, the Physicians Committee for Responsible Medicine, and the EPA developed webinars to provide overviews of both the CATMoS tool and the ICE database (ThePSCI.eu/training-videos-webinars).

EURL ECVAM recommends using 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.[675] EURL ECVAM additionally investigated how to increase 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.[676-678]

In its "Guidance on Information Requirements and Chemical Safety Assessment", 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.[679] 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 animal testing pursuant to REACH Articles 13(1) and 25(1).[680] More information about ways to reduce the number of animals used to assess acute oral toxicity for REACH can be found at ThePSCI.eu/training-videos-webinars.

[670]Kleinstreuer NC, Karmaus AL, Mansouri K, Allen DG, Fitzpatrick JM, Patlewicz G. Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation. *Comput Toxicol*. 2018;8:21-24.

[671]NICEATM. Predictive models for acute oral systemic toxicity. 2018. Accessed 10 December 2024. https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/acute-systemic-tox/models/index.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tox-models.

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[677]Prieto P, Kinsner-Ovaskainen A, Stanzel S, et al. The value of selected *in vitro* and *in silico* methods to predict acute oral toxicity in a regulatory context: Results from the European Project ACuteTox. *Toxicol In Vitro*. 2013;27(4):1357-1376.

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[679]ECHA. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 6. Published July 2017. doi:10.2823/337352.

[680]Commission Regulation (EU) 2016/863 of 31 May 2016 amending Annexes VII and VIII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards skin corrosion/irritation, serious eye damage/eye irritation and acute toxicity. http://eur-lex.europa.eu/eli/reg/2016/863/oj.

Dermal Route

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 scientifically sound justification for why the acute oral test results are protective for potential acute dermal effects.[681,682] In addition, dermal studies are not required for substances that are non-classified by the oral route and not absorbed dermally.[683] Furthermore, substances not classified by the oral route do not require dermal data under REACH Annex VIII.

[681]EPA OPP. Guidance for waiving acute dermal toxicity tests for pesticide formulations and supporting retrospective analysis. Published 9 November 2016. Accessed 25 August 2022. https://www.epa.gov/sites/production/files/2016-11/documents/acute-dermal-toxicity-pesticide-formulations_0.pdf.

[682]EPA OPP. Guidance for waiving acute dermal toxicity tests for pesticide technical chemicals and supporting retrospective analysis. Published 31 December 2020. Accessed 25 August 2022. https://www.epa.gov/sites/default/files/2021-01/documents/guidance-for-waiving-acute-dermal-toxicity.pdf.

[683]OECD. Guidance document on considerations for waiving or bridging of mammalian acute toxicity tests. OECD Series on Testing and Assessment, No 237. Published 13 April 2017. Accessed 17 January 2025. https://doi.org/10.1787/9789264274754-en.

Inhalation Route

Testing by the inhalation route can be avoided based on physicochemical parameters (e.g. low volatility) or if exposure through inhalation is unlikely (e.g. in cases in which the substance is not aerosolised or otherwise made respirable under conditions of use). When testing is required, non-animal methods can be applied to fulfil the informational requirements. For example, to fulfil an informational need, the EPA accepted the use of an *in chemico* biosolubility test, which showed that a polymer, initially classified as a poorly soluble, low toxicity substance, was soluble in simulated epithelial lung fluid and, therefore, was not a hazard concern from lung overload.[684] In another example, the EPA accepted data from *in silico* computational fluid dynamic modelling and *in vitro* testing using three-dimensional reconstructed human lung tissues to fulfil the re-registration requirements for a pesticide instead of a 90-day rat inhalation study.[685,686] Several other promising research efforts are underway to develop non-animal methods for inhalation toxicity.[687]

PETA Science Consortium International has hosted numerous webinars (ThePSCI.eu/inhalation-webinars) and workshops, at which several approaches were presented that could eventually replace animal testing for this endpoint.[688,689] Additionally, the Science Consortium has funded method development and organised several awards to provide researchers with equipment and *in vitro* respiratory tissues to conduct inhalation toxicity studies.[690] More information on inhalation toxicity testing can be found at ThePSCI.eu/our-work/inhalation.

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[685]EPA. Chlorothalonil: Revised Human Health Draft Risk Assessment for Registration Review. Published 21 May 2021. Accessed 25 August 2022. https://www.regulations.gov/document/EPA-HQ-OPP-2011-0840-0080.

[686]OECD. Case study on the use of an integrated approach for testing and assessment (IATA) for new approach methodology (NAM) for refining inhalation risk assessment from point of contact toxicity of the pesticide, chlorothalonil. OECD Series on Testing and Assessment, No 367. Published 1 September 2022. Accessed 17 January 2025. https://one.oecd.org/document/env/cbc/mono(2022)31/en/pdf.

[687]Clippinger AJ, Allen D, Behrsing H, et al. Nonanimal approaches to assessing the toxicity of inhaled substances: Current progress and future promise. *Appl In Vitro Toxicol*. 2018;4(2):82-88.

[688]Clippinger AJ, Allen D, Jarabek AM, et al. Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report. *Toxicol In Vitro*. 2018;48:53-70.

[689]Clippinger AJ, Allen D, Behrsing H, et al. Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity. *Toxicol In Vitro*. 2018;52:131-145.

[690]Barosova H, Maione AG, Septiadi D, et al. Use of EpiAlveolar lung model to predict fibrotic potential of multiwalled carbon nanotubes. *ACS Nano*. 2020;14(4):3941-3956.

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.

Antibody Production

Affinity reagents such as antibodies are essential tools used in research to bind to a molecule to identify or influence its activity. Every year, millions 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 many 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. Several countries, including Australia, Canada, Germany, the Netherlands, Switzerland, and the UK, have restricted or banned the production of antibodies obtained via the ascites method because of animal welfare concerns.[691,692]

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 2015 *Nature* commentary, 111 academic and industry scientists called for an international shift to recombinant antibodies for increased reliability and reduced batch-to-batch variability in affinity reagents.[693] In addition, a 2015 *Nature* news feature reported that antibodies might be the laboratory tool most commonly contributing to the "reproducibility crisis".[694] In fact, poorly characterised and ill-defined antibodies were considered a primary cause of irreproducible research in a survey of preclinical studies that found that 47 out of 53 studies could not be replicated. 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.[695] This issue is not limited to monoclonal antibodies. Polyclonal antibodies, which are dependent on the animal used to produce the antibodies and, by definition, vary in their composition, cannot be consistently reproduced, leading to calls within the scientific community to phase them out of research completely.[696]

In addition to the lack of scientific reliability and animal welfare concerns, there are significant economic issues related to using animal-derived antibodies. An estimated $800 million is wasted annually worldwide on unreliable antibodies.[697] 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, can be used in all traditional antibody applications, 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.[698,699] 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.[700,701]

International efforts have highlighted the importance of a large-scale transition from animal-derived antibodies to animal-free affinity reagents.

In its 2020 Recommendation on Non-Animal-Derived Antibodies, EURL ECVAM stated the following:

> *EURL ECVAM recommends that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic applications. […] EU countries should no longer authorise the development and production of antibodies through animal immunisation, where robust, legitimate scientific justification is lacking.*[702]

In the US, experts and organisations, including NICEATM and PETA Science Consortium International, are working to increase access to animal-free affinity reagents. In December 2019, both organisations convened a meeting to outline a pathway to improve the quality and reproducibility of research and testing by accelerating their production and use. The subsequent meeting report describes steps to overcome hurdles to a comprehensive shift from animal-derived to animal-free, sequence-defined affinity reagents.[703] More information on sources of animal-free affinity reagents, webinars, publications, and details of the scientific, economic, and ethical advantages of replacing animal-derived antibodies with animal-free options are available at ThePSCI.eu/our-work/antibodies.

Governments have the opportunity to advance science by committing to developing, producing, and importing animal-free antibodies and banning monoclonal antibodies produced via the ascites method. In 2022, the Recombinant Antibody Challenge was launched by PETA Science Consortium International, the Physicians Committee for Responsible Medicine, and the Alternatives Research and Development Foundation, offering grants for free catalogue recombinant antibodies for use in research and testing (ThePSCI.eu/funding/recombinant-antibody-challenge). In order to further expedite the replacement of animal-derived antibodies, we recommend providing additional grant opportunities for the generation and use of non-animal affinity reagents.

[691]Groff K, Brown J, Clippinger AJ. Modern affinity reagents: Recombinant antibodies and aptamers. *Biotechnol Adv*. 2015;33(8):1787-1798.

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[694]Baker M. Reproducibility crisis: Blame it on the antibodies. *Nature*. 2015;521(7552):274-276.

[695]Bradbury ARM, Trinklein ND, Thie H, et al. When monoclonal antibodies are not monospecific: Hybridomas frequently express additional functional variable regions. *MAbs*. 2018;10(4):539-546.

[696]Bradbury A, Plückthun A. Reproducibility: Standardize antibodies used in research. *Nature*. 2015;518(7537):27-29.

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[698]Groff K, Brown J, Clippinger AJ. Modern affinity reagents: Recombinant antibodies and aptamers. *Biotechnol Adv*. 2015;33(8):1787-1798.

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[700]Groff K, Brown J, Clippinger AJ. Modern affinity reagents: Recombinant antibodies and aptamers. *Biotechnol Adv*. 2015;33(8):1787-1798.

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Biological Drugs

Many vaccines and other biological drugs are produced or tested for quality, identity, safety, and efficacy in experiments that require large numbers of animals. These procedures often cause severe suffering before the animals die or are killed. Methods to produce and test these drugs without animals are increasingly available, but experience has shown that validation and regulatory acceptance of these methods have not guaranteed their use.[704-708] Activities intended to phase out the use of animals in this context must ensure that regulatory authorities and the industry commit to (1) transitioning to non-animal biological drug production platforms, (2) developing non-animal replacements for quality, identity, safety, and efficacy tests for all biological drugs, and (3) ensuring that non-animal methods are consistently used in place of animal-based tests whenever they are available.

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 their blood. These animal-derived immunoglobulins have disadvantages intrinsic to their animal origin, including the risk of adverse human immune response, high batch-to-batch variability, and the potential to transmit pathogens between species. Animal-derived antitoxins can be replaced with recombinant human antitoxins expressed in cell culture. Several recombinant antibodies have been marketed,[709,710] and more are in development,[711] including candidate therapeutic human recombinant antibodies created with funding from PETA Science Consortium International.[712,713]

With adequate funding and support from regulators, all biological drugs of animal origin, including antibodies (described above), can and should be replaced to resolve issues inherent in using antibodies derived from animals.

Non-animal tests for assessing quality are available, but no formal mechanism exists to ensure that barriers to their implementation are resolved in a timely manner.[714] 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).[715] 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).[716] Without 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, which is prohibitively expensive and slow. As a consequence, the industry's adoption of non-animal methods remains limited.[717] Additional barriers to the implementation of currently available alternative tests have been discussed at length in workshops and the literature for many human and veterinary biological drugs.[718-720] Accelerating and standardising processes that facilitate the use of these existing replacement methods is crucial.

Leadership from regulators that ensures coordination among international regulatory bodies and the industry on best practices will remove these barriers. Authorities must establish harmonised manufacturing consistency requirements, as tightly controlled manufacturing consistency policies are the foundation of many animal replacement strategies.[721,722]

[704]Dozier S, Brown J, Currie A. Bridging the gap between validation and implementation of non-animal veterinary vaccine potency testing methods. *Animals (Basel)*. 2011;1(4):414-432.

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[717]Veterinary Medicines Directorate. Animal usage in quality control tests for the batch release of immunological veterinary medicinal products (IVMPs) via the UK from 2007 to 2012. London: VMD; 2016. Published 2016. Accessed 25 August 2022. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/438916/_518852-v8-Animal_Usage_for_QC_Batch_Release_of_IVMPs_2007-2012.pdf.

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Foetal Bovine Serum

Foetal bovine serum (FBS) is a supplement for cell culture media that provides an undefined mixture of macromolecules that 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.[723,724] Because the unborn calves are not anaesthetised at the time of blood collection, they likely experience pain. In 2007, it was estimated that 600,000 litres of FBS were produced globally each year, which translates to the use of up to 1.8 million bovine foetuses for this purpose.[725] Given the significant increase in the use of cell culture for research and testing, the number of foetuses used is expected to substantially increase.

Several scientific concerns are associated with the use of FBS: (1) batch variation leads to reproducibility issues for *in vitro* studies using FBS (or other undefined animal-derived products such as bovine pituitary extract); (2) the unknown composition of the serum may complicate the analysis of data obtained from cultured cells and reduce human relevance leading to potentially unexpected or undesirable outcomes; and (3) risk of contamination by animal proteins or pathogens is especially problematic in the manufacture of biological drugs for human therapies.

Chemically defined, serum-free media or human platelet lysates can replace FBS in cell culture media. For optimal definition and reproducibility, a chemically defined, animal-free medium that avoids all animal-derived supplements should be used. Scientists have published workshop proceedings for more than 20 years calling for the transition from FBS to animal component–free and chemically defined media. [726-730]

Animal-free and chemically defined serum-free media are available for some cell types. For others, researchers may still need to optimise the concentration of supplement to replace FBS. Medium providers can assist researchers in finding the right animal component–free medium. Researchers are also working towards developing animal component–free media that can work across cell types.[731] Information on replacing FBS in cell culture media and developing serum-free media and listings of companies offering FBS-free products are available on PETA Science Consortium International's website (ThePSCI.eu/fbs) and in the Fetal Calf Serum–Free Database (https://fcs-free.org). PETA Science Consortium International has funded the transition of commonly used lung cell lines to cell culture media without animal-derived products.[732]

Government and regulatory agencies should move expediently to restrict the production and use of FBS and prioritise the development and use of non-animal media and supplements. Funding organisations should also provide funding for the transition of cells to available non-animal media and for developing and optimising non-animal, serum-free media when needed. In addition, any research project proposal application should include a section on whether animal-derived products (including serum) will be used and, if animal-derived products are used, details on the researcher's search for non–animal derived products and an explanation for why they were not able to be replaced for that project.

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