Research Modernisation Deal

Eine Strategie zur Modernisierung der Forschung und zum Ausstieg aus Tierversuchen

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PETA People for the Ethical Treatment of Animals • PETA Deutschland e.V.

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PETA Deutschland e.V. (PETA)

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 Forschungsförderern 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 Medikamenten-entwicklung, 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 Patienten. 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 übertragen lassen. Daneben beobachten wir auch die fortschreitende Entwicklung und Implementierung von Alternativ-technologien, 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 Gesundheit nicht vorangebracht hat. Der Einsatz von Tieren in diesen Bereichen sollte daher allmählich 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 Testverfahren.**

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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 jemanden kennt, der 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 Referenzlabor der Europäischen Union für alternative Methoden zu Tierversuchen (EURL ECVAM) 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 Grundsätze des 3R-Prinzips – Replace (Vermeiden), Reduce (Verringern) und Refine (Verbessern) – bei der Durchführung von Tierversuchen innerhalb 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.[6]

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.[7] 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.

i. Fehlende Aussagekraft

Probleme mit der internen und externen Validität tragen dazu bei, dass sich Erkenntnisse aus der biomedizinischen Forschung, die mittels Tierversuchen gewonnen wurden, nicht aus dem Forschungslabor in die klinische Anwendung am Patienten übertragen lassen. Die interne Validität von Tierversuchen wird durch ein schlechtes Studiendesign beeinträchtigt, beispielsweise wenn Tierversuchsleiter keine Maßnahmen zur Vermeidung von Voreingenommenheit implementieren, wie z. B. die Anonymisierung der Proben vor der Datenanalyse. Nach einer Meta-Analyse systematischer Reviews vorklinischer Tierversuche in verschiedensten Therapiebereichen stellten Wissenschaftler der Universität Oxford fest, dass der Nutzen von untersuchten Behandlungsmethoden aufgrund fehlender Maßnahmen zur Verringerung der Ergebnisverzerrung aus Tierversuchen wahrscheinlich überschätzt wird.[8] Die Autoren 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.“[9] Die Wissenschaftler 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.“[10]

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.[11]

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“.[12]

Aufgrund inhärenter Unterschiede zwischen Mensch und Tier können nichtmenschliche Tiere nicht als Analoga dienen, um die spezifischen biologischen Details, die zur Entwicklung sicherer und wirksamer Arzneimittel für den Menschen erforderlich sind, zu verstehen. Laut Wall und Shani können selbst „extrapolierte Ergebnisse von Studien mit zig Millionen Tieren die Reaktion beim Menschen nicht genau vorhersagen“.[13]

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.[14] Versuche, die Speziesunterschiede zu kontrollieren oder zu korrigieren, führen zu einem, wie die Autoren es nennen, „Extrapolator’s Circle“ (Extrapolationskreis): „Wenn wir feststellen wollen, ob die Wirkungsweiseeiner 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." Die Autoren befassen sich auch mit der besorgniserregenden Entwicklung unter Personen, die an Tierversuchen beteiligt sind, die Frage des Speziesunterschieds und die Auswirkungen auf die externe Validität zu verharmlosen – ein Problem, das jedoch von einer Reihe von Forschern durchaus anerkannt wird.[16,17] Wie Pound and Ritskes-Hoitinga weiter ausführen, ist es nicht verwunderlich, dass die Frage des Speziesunterschieds heruntergespielt wird, da sich die Experimentatoren ansonsten mit der „Möglichkeit auseinandersetzen müssten, dass das präklinische tierexperimentelle Forschungsparadigma nicht mehr viel zu bieten hat". Es besteht ein wachsender wissenschaftlicher Konsens darüber, dass mit humanrelevanten Forschungsmethoden und -technologien, die sich zur Lösung von humanbiomedizinischen und regulatorischen Bewertungsparadigmen besser eignen, weit mehr erreicht werden kann als mit Tierversuchen. Wie eine kürzlich veröffentlichte Branchenstudie hervorhob, ist es an der Zeit, die Entdeckung von Arzneimitteln und die Toxikologie zu humanisieren.[18] Dies ist besonders relevant in Deutschland, dem größten Biotechnologie-Markt nach den USA.[19]

ii. 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 Patienten übertragen lassen. Wie bereits erwähnt, versagen laut den NIH neue Medikamente „in rund 95 Prozent der Studien am Menschen"[20] – 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 Kollegen 101 Artikel, die in den renommiertesten medizinischen Fachzeitschriften veröffentlicht wurden und in denen die Autoren ausdrücklich erklärten, dass ihre Forschung zu neuen Anwendungsgebieten mit realistischem Potenzial für einen klinischen Durchbruch führen würde. Der Großteil der analysierten Artikel (63 Prozent) bezog sich auf Tierversuche.

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

Die Untersuchungen von Professor Ioannidis und seinen Kollegen 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.[21]

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.[22] Die Autoren weisen darauf hin: „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."[23]

Eine Maus in einem Labor reagiert auf ein Medikament nicht auf die gleiche Weise wie eine Maus in der Natur. Wenn sich Mäuse im Labor und Mäuse in der Natur schon dermaßen unterscheiden, wie soll eine Maus im Labor dann die Biologie des Menschen zuverlässig abbilden?

Die Schwierigkeiten bei der Übertragung von Ergebnissen aus Tierversuchen auf menschliche Patienten werden durch die Gefangenhaltung der Tiere und die unnatürlichen Bedingungen im Versuchslabor weiter verschärft, da diese das natürliche Verhalten der Tiere beeinträchtigen.[24] 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.[25,26,27,28,29] Darüber hinaus sind Tiere, die in Versuchslaboren ihre Physiologie und die Neurobiologie verändert haben, keine guten „Modelle" für ihre Artgenossen in der freien Natur. Eine Maus in einem Labor reagiert auf ein Medikament nicht auf die gleiche Weise wie eine Maus in der Natur. Wenn sich Mäuse im Labor und Mäuse in der Natur schon dermaßen unterscheiden, wie soll eine Maus im Labor dann die Biologie des Menschen zuverlässig abbilden?

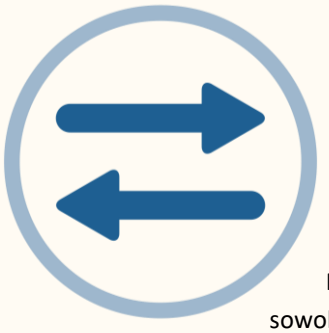
Beleg 1: Mangel an klinischem Erfolg

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. Forscher 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 Schlaganfallpatienten verbessert.“[30]

Onkologische Medikamente, die ebenfalls in Tierversuchen getestet werden, weisen eine Erfolgsquote von nur 3,4 Prozent auf.[31] 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.[32]

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 Forscher der US-amerikanischen Vanderbilt University den Einsatz eines evidenzbasierten Ansatzes, mit dem sich die Entwicklung nützlicher Medikamente für Patienten, 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.“[33]

Die Abkehr des allgemeinen Konsens von Tierversuchen lässt sich in verschiedenen

Bereichen beobachten, beispielsweise in Publikationen zur beschränkten Aussagekraft von Tierversuchen[34], in einer verstärkten Sensibilisierung der Gesellschaft für die kognitiven Fähigkeiten und die Empfindungsfähigkeit von Tieren[35] und in der rasant schwindenden öffentlichen Akzeptanz von Tierversuchen.[36] 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".[37] 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."

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.[38] Wenn die Forschungsfinanzierung ihre Prioritäten hin zu humanrelevanten Versuchsmethoden verlagert, können Patienten benötigte Behandlungen sicherer und wahrscheinlich in kürzerer Zeit erhalten.[39] 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

i. 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".[40]

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.[41]

Die Markteinführung eines neuen Arzneimittels kann über 2 Mrd. Euro kosten und bis zu 15 Jahre dauern.[42] 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[43], weil entweder unerwünschte Nebenwirkungen auftreten oder keine Wirksamkeit gegeben ist. Kacey Ronaldson-Bouchard und Gordana Vunjak-Novakovic, Wissenschaftler 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."[44]

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 Fehlverhalten und Fehlern bei der Veröffentlichung hin.[45] Auch im Deutschlandfunk[46] und im Laborjournal[47] 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.[48] Darüber hinaus werden auch in Fachzeitschriften, welche die ARRIVE-Richtlinien (Animal Research: Reporting of In Vivo Experiments)[49] 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.[50]

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.“[51]

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 Probanden im Rahmen der klinischen Phase-I-Studie eines französischen Auftragsforschungsinstituts oral verabreicht. Sechs Männer im Alter von 28 bis 49 Jahren litten an starken Nebenwirkungen und mussten ins Krankenhaus eingeliefert werden. Ein Teilnehmer 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 Probanden keine schädliche Wirkung festgestellt“.[52]

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 Probanden in lebensbedrohlichem Zustand. Da ein Multiorganversagen drohte, wurden sie auf die Intensivstation verlegt.“[53] Fünf der sechs Teilnehmer mussten nach der Anfangsdosis drei Monate im Krankenhaus bleiben, der sechste Proband lag im Koma. Selbst ein halbes Jahr später litten die Teilnehmer noch unter Kopfschmerzen und Gedächtnisverlust. Einem der Patienten mussten infolge einer Gewebs-Nekrose Zehen und Finger amputiert werden.[54] 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.“[55]

Doch auch das Gegenteil ist der Fall: Heilverfahren, die bei Tieren nicht wirksam waren, blieben ungenutzt und Patienten 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.[56] Forscher 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.[57]

ii. 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 2023 auf 29,3 Mrd. Euro anwachsen wird, und dass der Markt für induzierte pluripotente Stammzellen (iPSCs) und 3D-Zellkulturen im Jahr 2021 ein Volumen von 3,2 Mrd. Euro respektive 2,5 Mrd. Euro erreichen wird.[58,59,60] Die Marktforscher von BCC Research gehen zudem davon aus, dass der weltweite Markt für regenerative Medizin bis 2025 ein Volumen von 80,48 Mrd. Euro umfassen wird.[61]

Das in Boston, USA, ansässige Start-up Emulate, Inc. hat für die Erweiterung seiner Organchip-Technologie kürzlich weitere 32 Mio. Euro in Finanzierungsrunden eingeworben. Die Technologie wird derzeit von AstraZeneca, Roche, Merck, Johnson & Johnson und weiteren Unternehmen eingesetzt, um die Sicherheit und Wirksamkeit potenzieller Medikamente genauer vorherzusagen.[62] Auf europäischer Ebene finanziert das Projekt „Horizont 2020" die Entwicklung der nächsten Generation von Organ-Chips mit 18 Mio. Euro.[63] In Deutschland unterstützt das Bundesministerium für Bildung und Forschung die Entwicklung von Methoden zum Ersatz von Tierversuchen. Seit 1980 hat das Ministerium etwa 600 Projekte mit etwa 190 Mio. Euro finanziert.[64]

Beleg 3: Erneute Prüfung fehlgeschlagener Medikamente

Eine von Emulate und Janssen Pharmaceuticals im April 2018 veröffentlichte Studie zeigte, wie sich eine durch eine Antikörpertherapie verursachte Thrombose beim Menschen mit einem Blutgefäß auf einem Biochip vorhersagen lässt. Diese Antikörpertherapie war nach präklinischen Tierversuchen zuvor als sicher eingestuft worden. Die klinischen Studien mussten jedoch abgebrochen werden, nachdem die Probanden, denen das Medikament verabreicht worden war, Blutgerinnsel entwickelten, auf die es in den Tierversuchen keinen Hinweis gegeben hatten.[65]

Neue Technologien, wie die von Emulate entwickelte, werden die Dauer der Arzneimittelentwicklung verkürzen und den Prozess sicherer, billiger und effektiver gestalten. Die Entwicklung dieser Techniken ermöglicht 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 Wissenschaftlern 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.[66]

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".[67]

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".[68] Der Strategie zufolge könnten Fortschritte in den Bereichen Toxikogenomik, Bioinformatik, Systembiologie, Epigenetik und Computertoxikologie die Toxizitäts-prüfung 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 Forscher Modelle zur Beschreibung der Reaktionen auf Toxizitätspfade entwickeln sowie Modelle zur Abschätzung der erforderlichen menschlichen Exposition, um auf diesen Wegen Reaktionen hervorzurufen.[69]

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.[70]

Eine YouGov-Umfrage aus dem Jahr 2009, die in sechs EU-Ländern durchgeführt wurde, ergab eine überwältigende Ablehnung von Tierversuchen: 89 Prozent der Befragten aus Deutschland sprachen sich für ein Verbot aller Versuche aus, bei denen Tiere starken Schmerzen und Leiden ausgesetzt sind.[71] Daneben ist auch die öffentliche Unterstützung für Investitionen in tierfreie Testmethoden hoch: In einer von Ärzte gegen Tierversuche e.V. beauftragten Forsa-Umfrage unterstützten 69 Prozent der Befragten die Forderung, eine Strategie zum Ausstieg aus Tierversuchen in Deutschland zu entwickeln.[72] Außerdem befürworteten 74 Prozent der Befragten in einer von der britischen Regierung in Auftrag gegebenen Umfrage verstärkte Anstrengungen zur Entwicklung von Alternativen zu Tierversuchen.[73]

Angesichts der wachsenden Erkenntnis zur Empfindungsfähigkeit der Tiere ist der öffentliche Widerstand gegen Tierversuche nicht überraschend. Im Jahr 2012 unterzeichnete eine Gruppe anerkannter internationaler Neurowissenschaftler die sogenannte „Cambridge Declaration on Consciousness“. Darin erklärten die Forscher 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“.[74] 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

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?

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Mehr als 150 Wissenschaftler, Intellektuelle und Schriftsteller unterstützten zudem einen Bericht des Oxford Centre for Animal Ethics, der Tierversuche als ethisch und wissenschaftlich nicht vertretbar verurteilt.[75] „Der 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'", so die Autoren des Berichts. 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 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.[76] Die britische Gesundheits- und Sicherheitsbehörde (Health and Safety Executive) hat zudem Tierversuche für Pflanzenschutzmittel erheblich eingeschränkt.[77] In Deutschland untersagt das Tierschutzgesetz grundsätzlich Tierversuche zur Entwicklung von Tabakerzeugnissen, Waschmitteln und Kosmetika.[78]

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– Oxford Centre for Animal Ethics

Die niederländische Regierung gab Ende 2016 ihren Beschluss bekannt, Tierversuche für Toxizitätsprüfungen von Chemikalien, Lebensmittelzutaten, Pestiziden, Tierarzneimitteln und Impfstoffen bis 2025 einzustellen. Die Entscheidung fiel, nachdem das niederländische nationale Komitee für den Schutz von Tieren, die zu wissenschaftlichen Zwecken verwendet werden (NCad), die Notwendigkeit eines Paradigmenwechsels in Form einer Abkehr von Tierversuchen als Standardverfahren betonte. Der Bericht des Komitees zum Übergang der Niederlande zu einer tierfreien Forschung umfasste das Ziel, dass das Land im Bereich der tierfreien Innovation in der angewandten und translationalen Forschung international eine führende Rolle einnehmen soll.[79]

Die US-amerikanische Umweltschutzbehörde EPA kündigte 2019 an, die Zahl der Versuche an Säugetieren bis 2025 um 30 Prozent zu verringern und bis zum Jahr 2035 gar keine Tierversuche an Säugetieren mehr zu finanzieren bzw. zu verlangen. Zudem soll die Entwicklung tierfreier Methoden mit umgerechnet 3,8 Mio. Euro unterstützt werden.[80]

Solche Veränderungen sind erforderlich, damit die Qualität der biomedizinischen Forschung und der regulatorischen Bewertung verbessert wird und die Europäische Union sich als Weltmarktführer 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. Einige Länder, darunter die Niederlande, schreiben vor, dass systematische Überprüfungen durchgeführt werden müssen, bevor Tierversuche finanziert werden können. Wissenschaftler 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 Patienten.“[81]

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 Interessenvertretern 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.[82] 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“.[83] Die Autoren verglichen die Leiden, die Tieren in präklinischen Studien für sechs Behandlungsformen zugefügt wurden, mit den Vorteilen, die die Studien für den Menschen boten. 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.[84]

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“.[85] 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.

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.[86,87] 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, Verbraucher und internationale Handelspartner 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.

Nationale 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.

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Anhang (in Englisch)

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

Table of Contents

Glossary

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

Basic and Applied Biomedical Research

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

Cancer

Recommendation: End the use of animals immediately

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

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

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

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

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

Rodents are not suitable for radiation-induced carcinogenesis research, including for thyroid cancer. The nuclear architecture and spatial positioning of genes involved in radiation-induced injury are drastically different between

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

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

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

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

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

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

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

Cardiovascular Disease

Recommendation: End the use of animals immediately

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

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

Rodents are also resistant to atherosclerosis, a major cause of many cardiovascular diseases, owing to their lack of cholesteryl ester transfer protein.[25]

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

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

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

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

Diabetes

Recommendation: End the use of animals immediately

From 1984 to 2014, more than 50 papers were published per month describing experiments on rodent models of type 2 diabetes mellitus (T2DM).[37] Considering these numbers, we now know a great deal about diabetes, or metabolic disturbances that look like diabetes, in rodents, but "many details of human T2DM pathogenesis remain unclear, and means of preventing disease progression remain elusive".[38] Rodent studies were used to identify thiazolidinedione (TZD) drugs as possible therapeutics for humans with T2DM or insulin dysfunction. Unfortunately, the studies did not predict that TZDs would increase the risk of cardiovascular death in these patients by 64 per cent; in fact, they provided contradictory evidence.[39]

T2DM is a disease of glucose misregulation that leads to broad physiological effects. Rodents differ from humans on every tier of glucose regulation, from the level of nucleic acids to differences in proteins, pathways, cells, tissues, and organs. The two species also differ in terms of disease progression at the organism level and, dramatically, in environmental exposure and autonomy of lifestyle.[40,41] "Because mice rely principally on the liver for glucose homeostasis, while humans rely on skeletal muscle where transport mechanisms and biochemical pathways differ,

mice may not be expected to be analogous to [T2DM] patients in regards to mechanisms of glucose metabolism or its dysfunction."[42] Despite these clear discrepancies, diabetes research in animals continues while more relevant, human-based methods are often ignored.

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

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

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

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

HIV/AIDS

Recommendation: End the use of animals immediately

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

Because macaques are unreceptive to HIV, experimenters who wanted to use them shifted their focus to studying simian immunodeficiency virus (SIV), even though it is known that SIV isn't related to the most widespread HIV virus, HIV-1, but rather is a relative of the rarer and less pathogenic HIV-2.[51] The genetic homology between HIV and SIV is only 55 per cent, and SIV is less genetically diverse than HIV.[52,53] Owing to differences in surface proteins and other molecular markers, antibodies that neutralise SIV have no effect on HIV, and vice versa,[54] making them useless in HIV research. Importantly, the dose of SIV administered to non-human primates in experiments is much higher than the

typical amount of HIV-1 to which a human is exposed during sexual transmission.[55] AIDS researcher Mark Girard has stressed, "Extrapolating from vaccine protection results in non-human primate [SIV/SHIV] studies to efficacy in man may be misleading."[56]

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

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

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

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

Considering the differences between an animal laboratory environment and human society, it is clear that animal experiments will never capture the complexity of this human disease. Animals used in experiments are kept in mostly pathogen-free conditions, and cofactors that may be present in human patients, such as other microbial infections, are absent, significantly altering the acquisition and course of the virus.[63] Additionally, researchers at Emory University in Atlanta state, "HIV persistence is a very complex virological and immunological phenomenon, with infection of several cell types in a wide array of anatomic tissues that are all regulated differently,"[64] and recognise that human *in vitro* models are needed to replicate this human disease and develop treatment. Thinking progressively about non-animal methods, UK scientists have said, "Existing animal models predicting clinical translations are simplistic, highly reductionist and, therefore, not fit for purpose," and that clinical attrition data "focusses the attention back on to early target selection/lead generation, but it also questions the suitability of current animal models with respect to congruency with and extrapolation of findings for human hosts".[65]

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

Recently, scientists from Australia, France, Italy, and the UK have been studying the immune cells of individuals called "HIV controllers", who can become infected with HIV but are able to control the virus's spread without any intervening therapy.[68] The hope is that immune cells from HIV controllers can be transferred to HIV-infected patients to help them fight the virus. This promising research is human-specific and requires human-specific testing methods.

As Nobel laureate Sydney Brenner declared, "We don't have to look for model organisms anymore because we are the model organism."[69] Similarly, in 2007, the associate editor of *The BMJ* stated, "When it comes to testing HIV vaccines, only humans will do."[70]

Inflammation and Immunology

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

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

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

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

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

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

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

Considering the obvious failure of mice as surrogates in the study of human immune systems, investment in human-relevant *in vitro* and *in silico* models is needed. Advances in data collection and computer analyses have allowed for

the development of human-relevant multiscale models that "can consistently integrate immunological data generated at several scales, and can be used to describe and optimize therapeutic treatments of complex immune diseases".[87]

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

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

Nerve Regeneration

Recommendation: End the use of animals immediately

Many neuroprotective agents have been developed that are successful in treating spinal cord injury (SCI) in animal models, but clinical trials have been disappointing. Neurologist Aysha Akhtar has described three major reasons for this failure: "differences in injury type between laboratory-induced SCI and clinical SCI, difficulties in interpreting functional outcome in animals, and inter-species and interstrain differences in pathophysiology of SCI".[92] In their systematic review of the use of animal models to study nerve regeneration in tissue-engineered scaffolds, Angius and colleagues noted, "The large majority of biomaterials used in animal models have not progressed for approval to be tested in clinical trials in spite of the almost uniform benefit described in the experimental papers."[93] The authors lamented the low quality of described animal experiments, in that necessary detail and rationale had been omitted, making it difficult to compare data.

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

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

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

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

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

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

Human-relevant methods for studying nerve injury and regeneration have been reviewed by a number of research groups and include human organoids, microfluidics, engineered human tissue scaffold moulds, bioprinting, and other *in vitro* uses of humans cells. *Ex vivo* models, such as those that use 3-D engineered scaffolds, bioreactors, neurospheres, and organoids, allow for more controlled studies on specific parameters than do animal experiments.[99] Bioprinting can use bioinks containing human cells and materials to construct heterogeneous tissue models in a single step and with great consistency,[100] an aspect of nerve regeneration research that has been particularly lacking in animal models.[101]

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

Neurodegenerative Diseases

Recommendation: End the use of animals immediately

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

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

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

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

Genetically modified mouse models of neurodegenerative disease exhibit an inconsistent range of pathological and behavioural phenotypes, in part because of the transgenes used, inconsistencies in transgene insertion and expression, and mouse background strains.[110] The most commonly used genetic mouse model of MND, the SOD1 model, is based on a gene that accounts for only 3 per cent of MND cases in the human population.[111] Literature reviews have concluded that findings from this model have not translated into any effective human therapy for MND, that "a biased estimation of treatment efficacy in animals may lead to unnecessary (and possibly harmful) clinical trials in humans",[112] and that "animal models are not an ideal system for studying [amyotrophic lateral sclerosis (MND)] or for developing drug therapies".[113] In PD, even non-human primate studies do not "constitute a valid scientific modality for the complete understanding of PD and for the development of future neuromodulation therapeutic strategies".[114]

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

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

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

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

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

For many years, animal experimenters have tormented monkeys, mice, dogs, and other animals in an effort to create drugs to treat these devastating diseases; however, since other animals don't get these human diseases, experimenters have manipulated their genomes in order to force certain symptoms. The results, after decades of tests, include more than 100 failed drugs, an untold number of animal deaths, and the continued suffering of human victims of the disease. For these patients, a switch to human-relevant methods is long overdue.

Neuropsychiatric Disorders

Recommendation: End the use of animals immediately

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

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

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

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

To quote Dutch animal behaviourists van der Staay, Arndt, and Nordquist, "If evidence accumulates that the intended goal/purpose cannot be reached, then one should consider abandoning further development of the model."[139] This group also points out that in all cases, "benefits must outweigh the ethical costs of the animals. These costs include pain and suffering, distress and death".[140] Funds should be allocated to more relevant, human-based experimental models, such as computational modelling using already well-defined biomarkers[141] and the use of patient-specific stem cells for personalised medicine, which "affords the ability to generate neuronal cell-based models that recapitulate key aspects of human disease"[142] and can be used in drug discovery. Complex diseases like schizophrenia are ideal disorders "to model through stem cell approaches due to … heterogeneous, complex genetics that are hard to recapitulate in animal models".[143]

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

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

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

Sepsis

Recommendation: End the use of animals immediately

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

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

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

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

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

The "gold standard" method of inducing sepsis in mice is through caecal ligation and puncture. However, mice's responses to this procedure vary depending on age, sex, strain, laboratory, the size of needle used, and the size of the incision, which makes results incomparable between laboratories.[159] In addition, the procedure causes the formation

of an abscess, whose effects may disguise or be disguised by the effects of the sepsis itself.[160] This means that an intervention that appears to be beneficial for sepsis may actually be beneficial only because of its effects on the abscess.

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

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

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

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

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

Stroke

Recommendation: End the use of animals immediately

According to researchers at the Institute for Stroke and Dementia Research in Munich, "More than 1000 neuroprotective compounds have been tested in rodent models with the aim to improve stroke outcome. … Indeed, many agents reduced brain damage (in most cases measured as decreased infarct volume) in rodent models of

experimental stroke. Out of these candidates approximately 50 neuroprotective agents were tested in more than 100 clinical stroke trials, but none has improved outcome in clinical stroke patients."[174]

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

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

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

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

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

Kaya and colleagues made the following observation:

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

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

Thanks to technological advances, including accurate 3-D representations of multiple neuronal cell types and structures of the human brain, researchers are able to overcome some of the previously limiting factors of human *in vitro* brain research. As part of a $70 million NIH programme, an interdisciplinary team of researchers at Vanderbilt University have engineered a blood-brain barrier-on-a-chip, which they are using to study human brain inflammation induced by various compounds.[186] Similarly, the Seattle-based biotechnology company Nortis was recently awarded a federal grant to develop its predictive preclinical living model of the blood-brain barrier as an alternative "to traditional pharmaceutical drug development testing on laboratory animals", which will "reduce costs and minimize clinical trial failures".[187] Disruption of the blood-brain barrier following a stroke[188] is a critical factor to consider in attempting to move a potential therapeutic compound from a patient's bloodstream to the brain. Scientists at the University of California–Irvine opine that "[blood-brain barrier]-on-a-chip models offer tremendous potential for recreating microvasculature in the laboratory that will allow controlled study of the mechanics of [blood-brain barrier] permeability and immune infiltration as they relate to the process of stroke",[189] particularly those that employ human cells, such as human induced pluripotent stem cells, which "can be used to create clinically relevant models for [central nervous system] disease".[190]

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

Substance Abuse

Recommendation: End the use of animals immediately

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

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

Third, serious flaws in experimental design of addiction experiments greatly skew interpretation of their results. In the human experience with drugs, the user chooses to consume the addictive substance. They choose it over other

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

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

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

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

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

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

Trauma

Recommendation: End the use of animals immediately

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

Trauma is extremely heterogeneous: patients differ in age, gender, ethnicity, medical history, alcohol and drug use, and the presence of other injuries, making the production of an appropriate animal model difficult,[212] if not

impossible. In studies of traumatic brain injury, all promising therapeutics identified in animals have failed in human clinical trials.[213] There is a significant amount of discussion regarding the limitations of animal models of trauma and haemorrhagic shock, which is summarised in this excerpt from a review by Combes:

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

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

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

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

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

Training and Forensic Enquiries

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

Forensic Sciences

Recommendation: End the use of animals immediately

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

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

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

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

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

Medical Training

Recommendation: End the use of animals immediately

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

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

Microsurgery Training

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

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

A systematic review of microsurgical training methods supported these findings:

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

Trauma Training

A study published by a US Air Force team compared the self-efficacy reported by military trainees taught emergency procedures on human simulators versus those taught using live animals and found equivalent results in both groups, concluding that "the belief in the superiority of animal training may just be a bias" and that "if the goal for trainers is to produce individuals with high self-efficacy, artificial simulation is an adequate modality compared with the historical standard of live animal models".[236] The lead author published a separate letter in the same medical journal stating, "We have entered into an age where artificial simulator models are at least equivalent to, if not superior to, animal models. … [T]he military should make the move away from all animal simulation when effective equivalent artificial simulators exist for a specific task. For emergency procedures, this day has arrived."[237]

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

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

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

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

Toxicity Assessment

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

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

Exposure-Based Assessment

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

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

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

Skin Irritation/Corrosion

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

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

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

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

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

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

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

Eye Irritation/Corrosion

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

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

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

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

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

The EPA currently accepts the use of *in vitro* methods for the determination of eye irritation and corrosion when classifying antimicrobial cleaning products and other pesticide products on a case-by-case basis, and it has published a guidance document describing the testing framework that industry can use for this endpoint.[250] Also, the agency, in collaboration with the Science Consortium, NICEATM, and industry members, is currently engaged in evaluating these methods for use with agrochemical formulations through a side-by-side comparison of *in vitro* and *in vivo* data. This project is expected to be completed in 2019.

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

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

Skin Sensitisation

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

The assessment of skin sensitisation involves measuring the likelihood that a substance will cause an allergic reaction if applied to the skin. In animals, such assessments have previously been based on applying a test substance to the shaved skin of guinea pigs or to the ears of mice, who are later killed. Fortunately, for industrial chemicals and drugs, the regulatory requirement to test for skin sensitisation can be fully replaced with a combination of *in vitro* and *in chemico* assays that each address a different key event in the adverse outcome pathway (AOP) for this endpoint.[252] The methods distinguish between sensitisers and non-sensitisers and are recommended to be used in an IATA.

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

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

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

Pyrogenicity

Recommendation: Immediately eliminate the use of animals for pyrogenicity assessment

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

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

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

Since 2010, the monocyte activation test (MAT) has been validated and included in the *European Pharmacopoeia* (*Ph Eur*) as a test for assessing pyrogen contamination.[264] It mimics the innate human fever response *in vitro*, exposing human whole blood or isolated human monocytes to test articles followed by tests to detect pro-inflammatory cytokines released during exposure, and it is compatible with drugs and medical devices.[265] It avoids the aforementioned problems with the RPT and LAL tests, and case studies document instances in which the MAT detected pyrogen contamination in products that had passed the RPT and LAL but caused fever in human patients.[266]

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

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

Tobacco and E-Cigarette Testing

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

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

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

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

Genotoxicity

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

Currently, the assessment of genotoxicity typically follows a step-wise approach, beginning with a core battery of *in vitro* tests that may be followed up by *in vivo* studies if the *in vitro* results are positive. The major endpoints that must be evaluated are gene mutation, structural chromosomal aberrations, and numerical chromosomal aberrations. In its "Strategy to Avoid and Reduce Animal Use in Genotoxicity Testing", EURL ECVAM recommends the Ames test to

identify gene mutations, combined with the *in vitro* micronucleus test to identify both structural and numerical chromosomal aberrations.[284] If a substance produces negative results in both tests, it can be categorised as having no genotoxic potential and no further testing is indicated. If a substance produces positive results in either test, certain regulatory applications currently specify *in vivo* tests as the next step. This is because while *in vitro* tests are highly sensitive, producing false negative results at a low rate, they are less specific, producing false positive results at a higher rate. The number of false positive results can be reduced by using p53-competent human cells, evaluating cytotoxicity based on cell proliferation, and testing at reduced maximum concentrations.[285] These considerations have been incorporated into recent revisions of OECD test guidelines.

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

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

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

Acute Systemic Toxicity

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

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

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

Acute Oral Toxicity

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

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

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

Acute Dermal Toxicity

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

Acute Inhalation Toxicity

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

Carcinogenicity

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

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

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

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

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

In its guidance document on the Bhas 42 CTA, the OECD recommends that it be used as part of a testing strategy rather than as a stand-alone assay. When combined with other information, such as genotoxicity data, structure-activity analysis, and toxicokinetic information, CTAs in general – and the Bhas 42 CTA specifically – can contribute to the assessment of carcinogenic potential and may provide an alternative to the use of *in vivo* testing.[325]

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

Endocrine Disruption

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

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

Its Toxicity Forecaster (ToxCast) ranks and prioritises chemicals using more than 700 high-throughput screening assays, which cover a variety of high-level cell responses and approximately 300 signalling pathways, as well as

computational toxicology approaches. Data have already been generated on thousands of chemicals of interest to the EPA.

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

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

Repeat Dose, Reproductive, and Developmental Toxicity

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

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

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

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

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

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

The EPA's National Center for Computational Toxicology is also exploring the potential for chemicals to disrupt prenatal development through the use of its virtual embryo model, v-Embryo™, which integrates *in vitro* and *in silico*

modelling approaches.[339] While the field is gradually moving towards IATA strategies in order to cover the majority of possible mechanisms, much more research is required.

Aquatic Toxicity Testing

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

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

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

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

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

Laboratory Production Methods

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

Biologic Drugs

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

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

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

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

Regulatory leadership will ensure international regulatory and industrial coordination on best practices to remove these barriers. Regulatory authorities must establish harmonised manufacturing consistency requirements, as tightly controlled manufacturing consistency policies are the foundation of many animal-replacement strategies.[361,362]

Antibody Production

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

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

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

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

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

An EU-wide ban on the *in vivo* production of monoclonal antibodies using the ascites method should be introduced, in line with the one that has been in place in the Netherlands for more than 20 years, and the EU should further move to eliminate the import of animal-derived monoclonal antibodies and the use of animals in the hybridoma method.[372] In order to expedite such a ban, we recommend that member states and research funding bodies provide grant opportunities for the generation and implementation of non-animal affinity reagents.

Foetal Bovine Serum

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

Foetal bovine serum (FBS) is a supplement for cell culture media that provides an undefined mixture of macromolecules that function to maintain cell viability and facilitate cell metabolism, growth, proliferation, and spreading in culture. When pregnant cows are slaughtered, a large-gauge needle is used to draw the blood from the beating heart of the foetus. Because the unborn calves are not anaesthetised at the time of blood collection, they likely experience pain. It has been estimated that 600,000 litres of FBS are produced globally each year, which translates to the use of up to 1.8 million bovine foetuses for this purpose.[373]

Additionally, a number of scientific concerns are associated with the use of FBS, including batch variation leading to reproducibility issues for *in vitro* studies using FBS, the unknown composition of the serum, and the risk of contamination by animal proteins or pathogens, which is especially problematic in the manufacture of biologics for human therapies. Dutch organisations hosted workshops in 2003 and 2009 that called for the transition from FBS to non-animal serum supplements in cell culture.[374,375] A third workshop on FBS and alternatives was held in 2016, organised by the SET Foundation and the Deutscher Tierschutzbund (German Animal Welfare Federation).[376] The workshop report recommends increased funding and continued development of serum-free culture models and the use of serum-free media when establishing new cell lines. Because a universal chemically defined serum-free culture medium is not yet available and there is high demand for different cell types, the report recommends the use of human platelet lysate (hPL) as a replacement for FBS when a serum-free medium is not available.

Animal component–free and chemically defined serum-free media are available for some cell types. For others, researchers still need to optimise the concentration of each supplement to replace FBS. For these cell types, hPL, which is obtained from donated human platelets, contains growth factors essential for cell growth and proliferation and is a superior alternative to FBS for culturing cells. Listings of commercially available products and FBS-free media recipes published in scientific literature are available on the Science Consortium's website (PISCLtd.org.uk/fbs) and in the Fetal Calf Serum-Free Database (https://fcs-free.org/).

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

Scientific Advisory Capabilities of PETA and Its International Affiliates

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

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

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

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