Laboratory animal of the year 2023:

The mouse in autism research

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Since 2003, the Federal Association of People for Animal Rights has named the "Laboratory Animal of the Year". With this, we could celebrate the 20th anniversary of our project, if the topic were not so sad. The aim of our laboratory animal is to make animal experiments on a specific species public. We show what kind of suffering is inflicted on animals in the laboratory and discuss what animal-free options already exist. The year 2023 is dedicated to the mouse in autism research. This is only one of many research areas, as almost three quarters of all animal experiments are carried out on mice. This species has thus been the frontrunner for many years.

Menschen für Tierrechte
Bundesverband der Tierversuchsgegner e. V.

Content

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Photo: Pixabay/Jonathan Rieder

Welcome address:

We need a paradigm shift, not the modification of obsolete systems!

This year's patron of the "Laboratory Animal of the Year" is Mrs. Petra Martin, founder and Chairwoman of the foundation "Stiftung Zukunft jetzt!". Under the theme "Future is not a blow of fate, but the consequence of the decisions we make today", the trust foundation is dedicated to a sustainable, social as well as ecological transformation of society and the economy. This fundamental paradigm shift also includes our treatment of animals, including those who suffer hidden behind the walls of experimental laboratories.

In my private life as well as in my foundation, I am vehemently committed to showing our fellow creatures more empathy in all areas. Especially the animals, whose misery is hidden every day in the anonymity of testing laboratories.

That is why I am also engaged in non-animal and humane research methods. In doing so, it is not enough for me to modify "wrong" and obsolete systems. What we need is a paradigm shift away from the supposed gold standard of animal testing to modern, human-oriented research.

Autism research on genetically modified mice is a good example illustrating this. A complex disorder such as autism cannot be researched in artificially diseased animals.

There are now countless animal-free possibilities. Human cell cultures, multiorgan chips as well as computer simulations are not only ethically correct, they also have the decisive advantage of delivering results that are relevant to human beings.

The money that is still being spent on such absurd research projects should instead be invested in promising and future-oriented human-specific methods with the aim of completely replacing animal testing by reliable new methods. These are the positive visions of the future that we need.

Petra Martin

www.stiftung-zukunftjetzt.de

Petra Martin,
Founder & Executive Director
of the „Stiftung Zukunft jetzt!",
with family dog Feebee.
Photo: Private

Presumed native wood mouse (Apodemus sylvaticus) that occurs in many habitats in nature, especially with open herbaceous and shrub layers. Photo: iStock/CreativeNature_nl

Laboratory animal of the year 2023: The mouse in autism research

Since 2003, the federal association Menschen für Tierrechte has named the "Laboratory Animal of the Year". This annual nomination brings an animal species out of the laboratories' anonymity, makes the experiments on these animals public, uncovers disruptive factors in the development of animal-free methods and calls for their elimination. Through discussion, the Laboratory Animal of the Year should help to achieve the abolition of animal experiments as quickly as possible.

Mood disorder autism: No uniform clinical picture

Within the research community, there is controversy about what exactly autism is. For example, the term "autism" includes autistic disorder, Asperger syndrome, atypical autism, and "other phenomena." Since the forms overlap and different degrees of severity can occur, the generic term "autism spectrum disorders" is used.[1] Many scientists consider autism to be a neurodevelopmental disorder because of observed subtle, early-onset differences and abnormalities in brain development.[2] Different brain areas are linked differently in autistic people compared to those of non-autistic people.[4] Depending on its severity, the spectrum of symptoms ranges from difficulties in social interaction, hypersensitivity to sensory stimuli or motor problems to impaired speech and mental retardation.[4] It is understood that there is a profound developmental abnormality that underlies complex disorders of the central nervous system – especially in the area of perceptual processing. It begins in infancy.[5] In addition to impaired social interaction, there may occur peculiarities in communication as well as stereotypical and repetitive behaviors or special interests.[6]

Illustration: iStock/lemono

The prevalence of autism spectrum disorders is estimated to be 0.6 % – 1 % worldwide. Boys are four times more likely to have such a disorder than girls.[7]

For some time, however, autism researchers have been disputing among themselves about terminology in scientific publications, as well as at conferences and in social media. It seems unclear whether autism is a "disorder," a "disability," or merely a "difference," depending on its manifestation.[8] People who have mild manifestations, as in Asperger's syndrome, do not consider themselves to be ill, but simply "non-neurotypical", and consequently do not seek any treatment.[4] Other people, however, may be suffering.

As other neuropsychiatric disorders such as ADHD, anxiety, and obsessive-compulsive disorder may co-occur; it is difficult to identify the core phenomena that characterize autism. Predominantly, the disorder is thought to be genetic, with a smaller degree of environmental conditions also playing a role.[2, 3]

In more than 90 % of all cases, the disorder is said to be hereditary.[9] In genetic studies of autism spectrum disorders, more than 100 high-risk genes have already been identified, and it is estimated that several hundred more such genes will follow in the future.

Scientists estimate that more than 1,000 genes will be identified in future that mediate lower susceptibility to autism.[10] Genetically striking is a structural copy number variation in the genome that is thought to lead to a predisposition for autism.[2] Researchers have taken advantage of this observation to breed numerous autism animal "models."

In some cases, mutations are present in genes encoding cell adhesion molecules such as neuroligin and neurexin. It is hypothesized that these two proteins may play a role in synapse formation and in retrograde information transport during learning processes.[11] However, there is not one single "autism gene"; instead, a variety of different genomic regions are involved. Studies also suggest that especially epigenetic changes influence the risk for diseases. Prenatal maternal exposure during pregnancy to pesticides, polychlorinated biphenyl (PCB) pollutants, heavy metals, particulate matter, as well as maternal illness, an infection, or the use of certain medications have been discussed.[3, 4]

The mouse in nature and in the laboratory

All of the laboratory mice used in science are said to be descended from the house mouse (Mus musculus). Because breeding leads to genetic uniformity, the mouse genome in the laboratory is less variant than that of the mouse in nature. In contrast to the wild type, only one variant of most genes is supposed to be present. The strains also differ from the wild type in their coat color, fluctuating size as well as weight.[12]

Mice sleep in the nest.
Photo: iStock/Adrian Eugen Ciobaniuc

Scientists conduct research with mice because they are small, easy to keep, and have a rapid reproduction rate with a high number of offspring. Because of its short lifespan of two to three years, the complete life cycle can be studied much faster than in humans, for example, according to the Max Planck Society.[13]

In nature, mice live in fixed social associations; they need constant interaction with their conspecifics. Communication takes place via scents and sounds – especially in the ultrasonic range.[14] Studies have shown that when male mice encounter females in the ultrasonic range between 30 and 110 kilohertz, they present regular songs with different syllables and repetitive sequences.[15] In comparison, the upper hearing threshold of humans is about 20 kilohertz.

Laboratory mice are raised in cages.
Photo: iStock/unoL

In the laboratory, however, the life of a mouse is quite different. Mice are rapidly reproduced and kept in large numbers in a small space. According to Annex III, Part B of the European Animal Experiments Directive EU/63/2010, mice are only given an area of 60 to 100 square centimeters per animal at a height of 12 centimeters when kept in stock and during experiments in the laboratory.

A breeding pair is given an area of 330 square centimeters at a height of 12 centimeters according to the regulation.[16] The housing is not nearly animal-friendly, since the mice cannot fulfill their needs, e.g., for sufficient movement, climbing as well as gnawing.

Since scientists have increasingly looked at improving husbandry methods in the last few years, there is meanwhile at least litter in the small plastic cages, and sometimes there are little houses and employment material for the animals. In addition, there are apparently researchers who keep young, male mice in groups for reasons that are not explained in detail, which might stress them. This is due to the fact that dominant males show strong territorial behavior even in the absence of females. This can cause severe fights.[17]

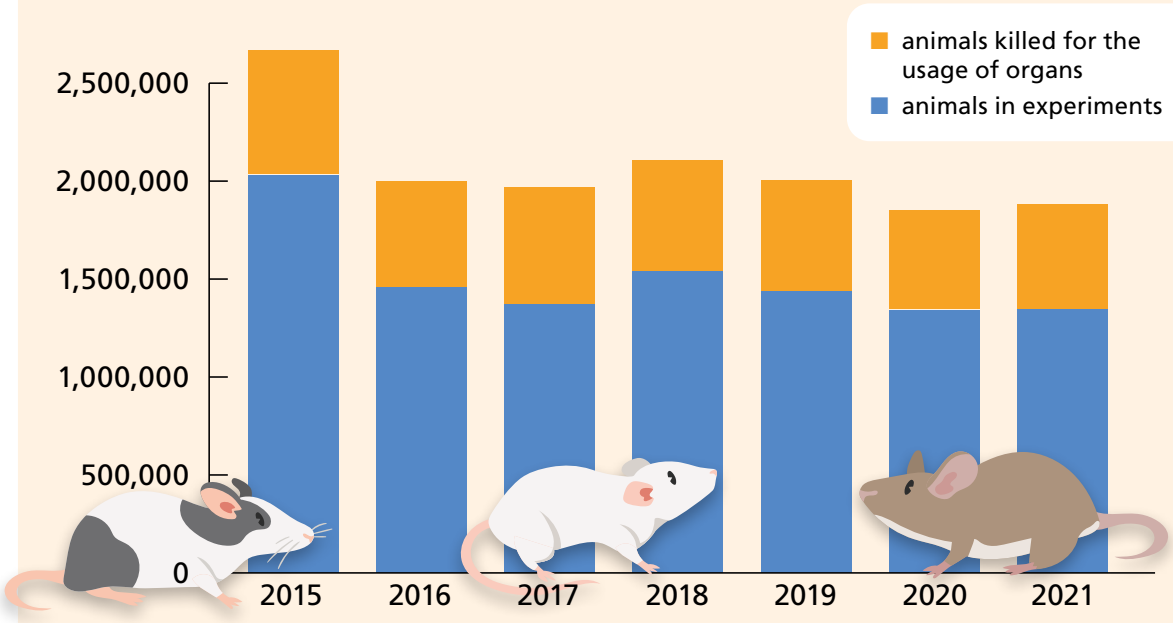
Figures

For decades, the mouse has been the No. 1 laboratory animal in Germany. In 2021 1,342,779 mice were used in animal experiments, representing 72.21 % of all laboratory animals. In recent years, the figures of mice used in animal experiments have stagnated. The number of mice killed for the removal of tissues and organs for research actually increased to 534,630.

In addition, 2,188,350 mice were bred for scientific purposes but then killed. Neither were they used in animal experiments nor, their organs/tissues were used for research purposes. Therefore, a total of 4,065,759 mice suffered alone in 2021. The number is so high, that it is difficult to comprehend. Among the more than 1.3 million mice in 2021, approximately 60 % were genetically modified, 13.7 % of them with a pathological phenotype[18], i.e., the genetic manipulation was associated with animal suffering. 4.7 % of all mice experiments were classified as severely distressing.

63.3 % of the experimental mice were used in basic research and 15.6 % in applied or translational research. The aim of the latter is to bring methods or therapies to the market as quickly as possible. Around 8.3 % of the mice suffered in legally mandatory tests. The remaining mice were used during education and training, or maintenance of breeding lines.

Animal experiments and killing for the removal of organs or tissues since 2015.



Mice killings without use in animal experiments were published for the first time in 2021. Therefore, they are not included in this diagram.

Illustrations: Freepik

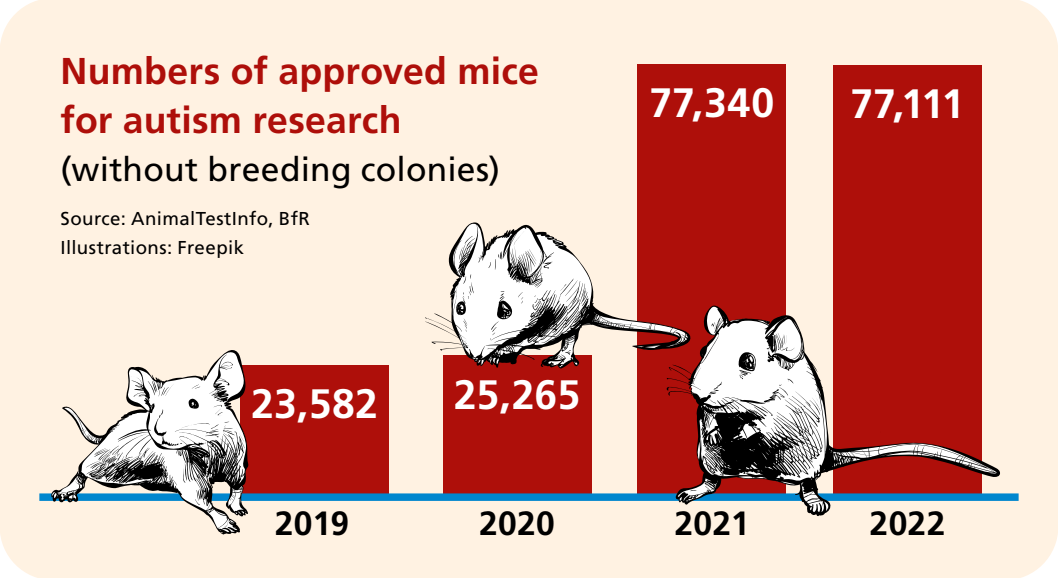
For the animal experiment, mice are kept in a standardized manner in small plastic cages. Source: iStock/ fotografixx

Mice in autism research

Official laboratory animal figures do not allow direct conclusions to be drawn about the number of mice consumed in autism research. The animal numbers are hidden in the category of "human nervous system research" and "mental disorders", which are likely to include autism disorders. For this reason, our estimates of animal numbers used for autism research are based on the non-technical project summaries (NTPs).

The NTPs record the approved animal experiments in Germany (not the actual consumption) and have to be published in a database of the Federal Institute for Risk Assessment (BfR) within 12 months.[19] The approved animal numbers are usually authorized for 3 to 5 years. The descriptions there are not always clear and often incomplete.

For some time now, the mouse has been the animal in autism research: while the numbers of approved mice were 23,582 in 2019 and 25,265 in 2020, they have already risen to over 77,000 animals in 2021 and 2022.[19] Several genetic causes of the neurological disorder autism have now been identified in humans, which researchers have used to breed different mouse models through genetic manipulation.



Typical genetically modified mice in autism research

Mutations that cause syndromal forms of autism disorder were identified over 20 years ago. Syndromal autism is the co-occurrence of autism in combination with a known syndrome such as fragile X syndrome. Here, a genetic cause is a possible reason for the occurrence of autism. On this basis, genetically modified mice have been produced that exhibit "similar" behavioral, cognitive, and physiological changes to those observed in patients.[20]

Examples:

- Fmr1+/y and -/y mice: mouse model of fragile X syndrome that has only some behavioral similarities to autism.[21] Through different signaling pathways, Fmr1 knockout in mice leads to learning and memory impairments.[20]
- Slc6a4 (+/- and -/-) mice: mouse model with manipulated serotonin transporter.[22]
- Igf-1 mice: insulin-like growth factor (IGF-1) can affect mTORC1 pathway regulating extracellular factors. Dysregulation of the mTOR signaling pathway is considered to be the major cause of several neurological disorders, including the autism spectrum.[23]
- (En2 -/-) Mice: the protein En2 (Engrailed-2) controls pattern formation during nervous system development. It is essential in the cell maturation of Purkinje fibers of the brain.[24]
- Dhcr7 (+/-) mice: Disruption of the enzyme 7-dehydrocholesterol reductase (DHCR7) leads to delayed growth and intellectual deficit in humans. Patients exhibit hyperactivity, auto-aggression, and sleep disturbances.[25]

Photo: iStock/toeytoey2530

Since most autism animal "models" only show autism-like behaviors, scientists are using humanized mice in which a human mutant or structural copy number variation has been integrated into the genome.[2]

To silence genes, mouse embryos are often taken out alive from the uterus of the pregnant female mouse and then genetically manipulated. Afterward, the embryos are returned to the body cavity. At specific developmental intervals, a number of dams are killed by breaking their necks to get the fetuses in order to examine their brains. Some of the offspring may be carried out entirely and will be used in the experiment. The genetically engineered mice are separated by sex, housed in plastic cages, and subjected to behavioral tests.[26]

As male mice emit ultrasonic vocalizations in response to the scent of a female in heat, and mouse pups communicate this way when physically separated from their mothers, researchers have interpreted a deficit in such vocalizations as an autism phenotype and equated it with communication deficits in autism patients.[20]

The tests include, for example, the Elevated Plus Maze (EPL). The model is based on the test animals' reluctance to spend time in open spaces and their tendency to move to the side where they think they will be safer. In the EPL, this fear is expressed by an increased time spent of the animal in the closed arms. Other tests include the activity in an open field, the rotarod, the three-chamber test, a social approach test, or a sense of smell test for buried food.[2, 26] The three-chamber test assesses the presence of a natural preference for social contact over the exploration of a new object.[20]

Photo: Wikipedia. CC BY-SA 3.0

Photo: Bmouzon, Creative Commons BY-SA 4.0

Left: Elevated Plus Maze. The Elevated Plus Maze test measures anxiety-like behavior. It is based on mice's natural aversion to open and elevated areas, as well as their natural spontaneous exploratory behavior in new environments. The apparatus consists of open and closed arms. The mice have access to all arms and can choose freely. The number of entrances to the open arms and the time spent in the open arms are taken as indications of courage in the open space.[27]

Right: Mice on or under the rotarod wheel. Three animals are already showing signs of fatigue and have fallen off the spinning axle.

Some researchers conclude that the behavioral deficits observed in the mice correspond to behavioral abnormalities observed in humans with autism spectrum disorders. However, other researchers acknowledge that the relationship between these behavioral abnormalities as well as underlying disturbances in neural circuitry and synaptic function is poorly understood. Additionally, different components of the autism spectrum may be associated with dysfunction in different brain regions. Furthermore, since results of behavioral tests are highly dependent on genetic background, environmental factors, and experimenter experience and skill, electroencephalograms and functional magnetic resonance imaging data are additionally necessary, according to resaerchers.[2]

To investigate environmental risk factors of autism, pregnant female mice are exposed to toxic chemicals (such as valproic acid). However, it is acknowledged that understanding of pathophysiological mechanisms in such models is limited.[20]

Researchers are also increasingly unconvinced of the mouse as an autism model: Therefore, with the help of the gene scissors CRISPR/Cas9, Javanese monkeys have already been equipped with gene mutations that also occur in some autistic individuals. Since the brain of macaques is more similar to that of humans, they are therefore more suitable for medical studies than mice.[4]

Photo: iStock/Iva Dimova

Criticism

Despite the numerous animal models, there is limited understanding of the underlying neurophysiology of autism, limited evidence of the efficacy of existing agents in autism populations, and limited translation of results for new drugs in animal/cell models to human studies.[28]

For example, social withdrawal is an indicator of depression, and may instead be attributed to the social communication difficulties that are characteristic of autism. Autistic individuals with concurrent psychosis may exhibit severe or manic depressive episodes and/or bipolar disorder with psychotic characteristics[28], which may be difficult to represent with an animal model. There is also an association between increased mood problems and increasing IQ in autism, which is thought to be related to greater awareness of one's difficulties.[28]

Since it is also thought that chemicals may be relevant that could have an effect on the developing brain of the child, the hormonal or immune system[7], there is a reflexive tendency to call for animal testing. However, developmental neurotoxicity testing can now be performed in an animal-free testing strategy that is far more relevant. With regard to endocrine disruptors, work is underway on meaningful non-animal methods (NAMs), as animal testing is not a good evaluation method for the protection of human health and the environment. Hormone efficacy is not a simple toxicological endpoint with a defined threshold – it is a dynamic and turbulent system that is inherently difficult to measure due to its inherent variability. The endocrine system consists of a complex set of interrelated hormones that regulate numerous fundamental biological processes. Therefore, it is not possible to simply test a substance for hormone activity in an animal experiment. A hormone-active substance could have multiple effects on brain development, but also on, e.g., sugar metabolism. In addition, unrealistic high doses and stress that the animals have to suffer, for example, through stomach tubes, can lead to unusable results.[29]

Alternatives


Photo: iStock/CreativeNature_nl

New studies on the molecular pathophysiology of autism using, for example, induced pluripotent stem cells[30] or in vitro disease models offer opportunities for drug screening and disease diagnostics. Effects of environmental chemicals and their mixtures on the developing brain can be better assessed using in vitro neuronal cell cultures or organ-on-a-chip technologies, in combination with, for example, placental tissue.

Here are some examples of research using animal-free methods:

1. Through magnetic resonance imaging (MRI) studies, a team of French researchers has discovered a brain structure typical of autism: a less developed fold of the Broca's area. Broca's area is responsible for language and communication – functions that are impaired in a certain autism variant. They studied boys with and without autism spectrum disorders aged 2 to 10 years and found that the brain furrow was significantly less pronounced in autistic children than in the control group. They concluded that communication skills may be lower the flatter the Broca's furrow is developed.[31]

2. A team of researchers from Austria, Italy, and the U.S. has developed brain organoids from patient cells as well as healthy volunteers for comparison to better understand the causes of autism spectrum disorders in early brain development. The team was able to observe how mutations in the CHD8 gene disrupt developmental processes that are characteristic of autism.[32] The gene encodes a chromodomain helicase DNA-binding protein (chd8). Among other things, it is involved in promoting cell proliferation and regulating RNA synthesis. Different expressions of this gene are associated with autism spectrum disorders.[33]

3. Using a pluripotent human stem cell-derived model of early brain development, scientists succeeded in demonstrating that relevant therapeutic concentrations of an antidepressant (paroxetine) induce abnormalities in brain cell development that could lead to adverse effects in developing offspring in the womb.[34]

4. Cell cultures consisting of the cell types neurons and astrocytes are suitable to investigate substance mixtures for their developmental neurotoxicological effects. In doing so, scientists took advantage of the key events of an Adverse Outcome Pathway (AOP) network.[35] In the concept of AOPs, biological events such as cell receptor binding of molecules lead to adverse effects in the organ, organism, or population through multiple sub-steps.

5. Artificial intelligence (AI) can be used not only to diagnose the disease, such as FreeSurfer[36] but also to identify new biomarkers. (37) Deep learning technologies can be embedded into existing autism screening to assist stakeholders in the early detection of autism traits.[38]

Since DNA methylation is the most extensively studied epigenomic mechanism, it can be used for genome-wide methylation analysis using placental tissue. For this purpose, AI and deep learning platforms have been used to identify and evaluate methylation markers for autism

detection. The researchers succeeded in predicting autism with a very high probability and reliability.

Using so-called Ingenuity Pathway Analysis (IPA), a web-based bioinformatics application that allows data analysis results from high-throughput experiments, such as microarray as well as RNA-Seq gene expression, miRNA, SNP, metabolomics, proteomics data, and Next Generation Sequencing, which can be uploaded and functionally analyzed, prenatally dysregulated molecular pathways associated with autism could be revealed.

This allows to identify and better understand genes and molecular pathways that are dysregulated in autism. IPA can also be used to find information about genes, proteins, and the molecular effects of chemicals and drugs.[40]

Assessment/Outlook

Although behavior cannot be displayed in vitro, the basics of genetic and epigenetic changes should initially be explored in vitro in combination with the identification of signaling pathways using AI and Deep Learning platforms. Only when the autism spectrum can be classified a therapy can be considered at all.

Animal testing is difficult because many disorder "patterns" can only be clarified by communicating with the affected persons. The corresponding communications with animals are very difficult, too coarse and error-prone.

At present, there are hardly any treatment options; one perspective would be the approach of an integrated testing strategy with in vitro/in silico methods, similar to what has already been developed for developmental neurotoxicity.

Implementing a package of measures

The Federal Association People for Animal Rights (Bundesverband Menschen für Tierrechte) campaigns for the abolition of animal experimentation on a scientific, political as well as social level. The Laboratory Animal of the Year is a tool with which the association informs the public and points out concrete possible solutions. To achieve its goal, the organization has compiled a catalog of measures to phase out animal experimentation and is calling on policymakers to develop an overall strategy for animal-free science. Right at the top of the list of necessary measures is the massive expansion of animal-free research, in particular by increasing research funding within Germany and the EU.

Equally indispensable are new criteria for the allocation of funding and the promotion of young scientists. Therefore, the establishment of chairs and professorships for animal-free science, teaching and training is an absolute must. At the level of regulatory approvals, it is necessary to enable a drastic shortening of the testing and approval times for non-animal methods. Currently, this phase takes between six and 15 years! Another important accompanying measure is, in particular, the ban on heavy-duty animal experiments without exception.

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Wir freuen uns, dass Sie sich für unsere Arbeit interessieren. Um die Abschaffung des Tierversuchs zu erreichen, sind wir als gemeinnütziger Verein auf Ihre Mithilfe angewiesen.

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Trotz Tierschutzgesetz und Staatsziel Tierschutz leiden jeden Tag Millionen Tiere in Tierversuchen, in der industriellen Landwirtschaft, auf Transporten und Schlachthöfen. Hinzu kommen artwidrig gehaltene Haus- und Wildtiere in Privathaushalten, in Zoo und Zirkus, „Pelztiere“ und unzählige Tiere, die jährlich Opfer der Jagd werden. Um dieses millionenfache Leid zu beenden, setzen wir uns aktiv für den Ausstieg aus dem Tierversuch und der „Nutztier“-Haltung sowie gegen jeglichen Missbrauch von Tieren ein. Um diesen Systemwechsel einzuleiten, brauchen wir einen Masterplan für den Abbau von Tierversuchen und eine Kehrtwende in der Landwirtschaft von der tierischen zur pflanzlichen Eiweißproduktion. Unser langfristiges Ziel: Das Mensch-Tier-Verhältnis muss sich grundsätzlich ändern. Tiere haben ein Recht auf Leben, auf Freiheit und auf Unversehrtheit. Der Weg zur Anerkennung dieser Rechte ist beschwerlich – wir gehen ihn pragmatisch, schrittweise und konsequent.

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Der Bundesverband ist seit über 30 Jahren als gemeinnützig und besonders förderungswürdig anerkannt. Spenden und Mitgliedsbeiträge sind steuerlich absetzbar.

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