Petition to the US Food and Drug Administration for Mandatory Use of Non-Animal Methods in the Development and Approval of Drugs and Devices

The Mandatory Alternatives Petition Coalition

(Short Name: Mandatory Alternatives Petition)

Official Version

November 14, 2007

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Purpose, Rationale and Scope

This petition seeks changes in US Food and Drug Administration (FDA) regulations and policies that would require, and not merely permit or recommend, that pharmaceutical companies, device manufacturers, and other entities regulated by the FDA submit data only from scientifically satisfactory non-animal test methods, and in lieu of corresponding animal test methods, whenever such scientifically satisfactory methods are available. Although there are sound scientific, economic and humane reasons why the use of scientifically satisfactory non-animal test methods should be mandatory, there is currently little incentive or support for researchers, businesses, regulatory agencies and educators to adopt them, or to develop new ones.

In contrast to the US, Europe has progressed in this area following European Union (EU) Directive 86/609/EEC in 1986. In particular, Article 7.2 states, "An experiment shall not be performed [on an animal], if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available." This requirement is legally binding on all EU member states. Adoption of a similar requirement in the US would go far toward harmonizing policy and practice on both sides of the Atlantic, as well as toward replacing animal use with scientifically satisfactory non-animal alternatives.

As set forth in this petition, the FDA has the authority to mandate the use of non-animal alternatives and to restrict the use of animal test methods for purposes of FDA approval. This petition requests that the FDA exercise this authority to promulgate a regulation mandating that investigators and testing facilities use scientifically satisfactory non-animal replacements for animal-based methods when meeting the requirements of the Federal Food, Drug and Cosmetic Act.

II. Current Practice: Advantages, Validation and Regulatory Approval of Alternative Methods

Non-animal test methods spare significant numbers of animals from pain and distress, are typically less costly and time-consuming, and may require lower investment in personnel and other resources. Most importantly, they often have more predictive value and specificity for the human condition. Examples of the superior predictive value of non-animal tests include the embryonic stem-cell test for embryotoxicity, new assays for skin corrosivity, in vitro tests for cancer causation and drug efficacy/toxicity at the US National Cancer Institute (NCI), and microdosing technologies. In fact, some 30 empirical studies have so far been published showing equal or greater efficacy for non-animal methods.

Animal test methods, many of which have been in use for decades, have never been scientifically validated. Despite this, a bias persists towards them, and with no true gold standard available they have typically been used as the default standard against which non-animal tests are judged. In vitro data (as an example) may show only 55-80 percent correlation with animal data due to the latter's natural variance, and so may *appear* inferior to animal tests due to the biological variability in the animal testing methods. In addition, human tissue-based in vitro methods may not show high

correlation with animal tests because they correlate better with the human response – which is, after all, the ultimate goal. These discrepancies demonstrate not any inferiority of the in vitro alternative methods, but rather the scientific fallacy of animal test results as the standards of reference for such methods.

More than two dozen alternatives to animal tests have been validated by the European Centre for the Validation of Alternative Methods (ECVAM), and at least ten methods and deletions have gained regulatory acceptance to date in the EU. In the US, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) has validated many fewer methods, clearly indicating a need for greater harmonization. This need is further illustrated by the favorable impact harmonization has had on reducing animal use in drug development and safety testing worldwide, by means of guidelines from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the Test Guidelines Program of the Organisation for Economic Cooperation and Development (OECD).

III. Current Practice: Costs and Problems Associated with Animal Use

Extrapolating research results across species is a tenuous enterprise. Animal test data are compromised by species differences in anatomy, organ structure and function, toxin metabolism, chemical and drug absorption, and mechanisms of DNA repair. These differences are compounded by additional variables related to experimental animal demographics and husbandry.

These problems manifest repeatedly when attempting to apply animal data to human diseases and drug responses. Examples include hormone replacement therapy for women, development of HIV protease inhibitors, Vioxx and other COX-2 inhibitors, teratology studies, harmful effects of smoking, non-steroidal anti-inflammatory drugs, antibiotics, antivirals, antidepressants, and cardiovascular medications, among others. Many harmful and ineffective drugs have tested safe and effective in animal studies. Conversely, many safe and beneficial human drugs would not survive animal testing today because of severe or lethal toxicities in some species.

Entire fields of translation science have demonstrated the failed paradigm of animal testing to predict human treatment responses. All of more than 80 preventive and therapeutic HIV/AIDS vaccines successful in nonhuman primates have failed in human trials. More than 4,000 studies have been reported demonstrating the efficacy of more than 700 treatments in animal models of stroke, yet none of the approximately 150 of these tested in humans has shown clinical benefit.

The entire field of cancer immunotherapy animal research has failed to produce even one successful therapeutic cancer vaccine. And dozens of human clinical trials have failed due to toxicities or lack of efficacy after animal tests showed cures, mitigation, or prevention of diseases such as diabetes mellitus, spinal cord injury, multiple sclerosis, psychiatric disorders, and many others.

Ninety-two percent of drugs that enter clinical trials following extensive animal testing fail to achieve FDA approval for marketing, and this failure rate is at least 95 percent for cancer drugs. Of the eight percent overall that are approved, half are

withdrawn or relabeled due to severe or lethal adverse effects not detected during animal testing. Levels of discordance between results from animals and humans range from 67 to 96 percent.

Recent examinations of animal trials have shown that they may occur concurrently or even after human studies, and the results are often conflicting; that there is poor correlation of cancer risk between assessments carried out by the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC); and that transgenic animal models frequently fail to duplicate human symptoms characteristic of many conditions, let alone enable scientists to elucidate the molecular processes underlying those diseases.

There are also economic advantages to replacement methods. For example, the DakDak test (used to measure the efficacy of sunscreens in preventing skin damage) can provide data for five or six products at less than half the cost of testing one product in animals. The current gold standard for testing a chemical to determine if it is carcinogenic is the rodent bioassay, which takes up to five years from planning to evaluation and review, at a cost of up to more than $4 million per substance. In vitro screening allows companies to identify promising test compounds in a cost- and time-efficient manner before progressing to expensive human trials.

Additionally, non-animal test methods save on various costs associated with animal methods, including animal procurement, maintenance and husbandry, and hazardous waste disposal. Finally, costly legal claims against companies that rely heavily on animal data may become more commonplace. For example, the pharmaceutical company Merck and Co., Inc. is currently facing litigation for alleged improper reliance on animal tests to show that its painkiller Vioxx was safe for humans.

Other factors resulting in the inherent suffering of animals used in testing include the manner in which animals are housed, transported and handled. Common laboratory routines have been shown to cause pronounced stress that can influence test results, and links between such stress and the development of behavioral stereotypies (which are believed to reflect animal suffering) are well established.

IV. Comparison of US and European Law

The potential for animals to suffer pain and distress in experiments was acknowledged in the US with the passage of the Animal Welfare Act (AWA) in 1966. Since its adoption, several amendments to the AWA, along with other supporting regulations and guidelines designed to improve the legal protection of animals in laboratories, have sought to reduce animal suffering in research and testing, and have contributed to the promotion of non-animal alternatives to the use of animals in drug and product testing, research, and education.

Noteworthy in this regard is the 1993 National Institutes of Health Revitalization Act (NIHRA). This law calls for the NIH to "conduct or support research into methods of biomedical research and experimentation that do not require the use of animals," as well as for reducing the number of animals used in research. In 1997, the U.S. Congress established ICCVAM, comprising representatives from 15 federal agencies. ICCVAM's purpose is to conduct evaluations of new, revised and alternative test

methods and to promote the scientific validation and regulatory acceptance of test methods that replace, reduce, or refine the use of animals.

Directive 86/609/EEC by the Council of the European Community is a primary factor responsible for Europe's pre-eminence in the field of non-animal methods, asserting that it is scientifically and morally insupportable to harm or kill animals when scientifically satisfactory alternatives are available. The EU Directive mandates the use of such non-animal methods, places their development and implementation into the political agenda of all EU countries, and has helped to spawn further legislation. The primacy of non-animal methods is largely accepted as the standard of practice by EU-based researchers and industry, but not yet by their American counterparts.

V. Support for this Petition

The American public is uncomfortable with animal experimentation and testing, particularly when it involves pain and distress or the testing of non-essential products. As a publicly funded agency, the FDA has a duty to use public monies in a cost-effective manner, and is obligated to address the concerns of the American public.

According to polls, 75 percent of Americans disapprove of animal experimentation and testing that cause severe pain and distress, and one-third object to all animal experimentation. Testing procedures account for the vast majority of animals reported in the highest categories of pain and distress, underscoring the importance of replacing animal use in regulatory toxicity testing. These prevailing sentiments suggest widespread public support for the goal of this petition: that replacement methods be *required* when they are available and proven scientifically satisfactory.

Broad scientific support for non-animal methods is demonstrated in the language drafted for adopted and proposed legislation, in the growth and diversification of such methods, and in the number of scientists and scientific organizations supporting them.

VI. Action Requested

The time is appropriate for regulatory and policy changes in the use of animals for preclinical drug and device testing. As long as FDA regulations and practices do not provide a mandate to achieve this, there is little incentive to change from the current suboptimal preclinical testing methods. Requiring the use of humane and scientifically sound alternatives is vital to improve the accuracy of preclinical testing, minimize the approval of hazardous drugs and devices, decrease pain and suffering to animals, and advance the goal of replacing animal tests with more reliable and humane methods.

We request the FDA to promulgate a regulation to mandate that an animal experiment should not be performed if another *scientifically satisfactory** method for obtaining the result(s), not involving the use of animals, is available. The specific actions requested of the FDA are detailed in **Section A** of this petition.

---

* *Scientifically satisfactory*: validated as an acceptable alternative to one or more animal tests currently in use, as evidenced by approval and enactment by any of the participant members of ICH (the US, the EU, and Japan), or by the FDA itself.

We further request that the FDA implement the objectives of the proposed regulation as follows:

A. The FDA should designate as *scientifically satisfactory* any and all methods validated as acceptable alternatives to one or more animal tests currently in use, as evidenced by approval and enactment by any of the participant members of ICH (the US, the EU, and Japan), or by the FDA itself.

B. The FDA should develop and implement standardized procedures requiring that its drug and device application reviewers accept as valid and sufficient any and all data submitted using scientifically satisfactory alternatives to animal test methods. Individual FDA reviewers must not require additional animal test data in such instances, or applicants will seek to include such data in initial applications so as to avoid approval delays.

C. The FDA should use the strongest possible language in its industry guidances regarding the acceptability and sufficiency of scientifically satisfactory non-animal alternatives, clearly indicating that the FDA requires only the non-animal data and does not require (and will not request) any animal test data thereby replaced.

D. The FDA should support efforts to obtain adequate funding directed specifically toward the development and utilization of its extensive human drug database as a particularly effective method for improving preclinical and clinical drug testing and public safety, informing FDA decisions regarding content of new drug or device applications, and replacing animal test methods with scientifically satisfactory non-animal alternative methods.

Petition to the US Food and Drug Administration for Mandatory Use of Non-Animal Methods in the Development and Approval of Drugs and Devices

A. ACTION REQUESTED

1. To promulgate a regulation that provides:

(a) In meeting the statutory requirements to demonstrate that a new drug or device is both safe and effective, pursuant to 21 U.S.C. § 355, applicants shall, to the greatest extent possible, rely on non-animal testing;

(b) For each application for a new drug or device approval submitted pursuant to 21 U.S.C. § 355, for which any animal testing is relied on, the applicant must certify that there was no non-animal alternative testing method available to make the necessary demonstration of safety and efficacy required by 21 U.S.C. § 355, and that any animal tests used afforded the maximum benefits for replacement, reduction, and refinement of animal use, as follows:

The undersigned, on behalf of the applicant for FDA drug or device approval, hereby certifies that the reason animal testing was used to supply information required by 21 U.S.C. § 355 was that, at the time the data were compiled, there were no scientifically satisfactory non-animal alternative methods available to obtain the data required by 21 U.S.C. § 355. The undersigned further certifies that any animal tests used afforded the maximum benefit of (i) first replacement; (ii) then reduction; (iii) then refinement of animal use available from scientifically satisfactory alternatives.

This certification is submitted pursuant to 28 U.S.C. § 1746.

(Signature) _____

(Name) _____

(Title) _____

(Address/Phone No.) _____

(Email Address) _____

(Date) _____

2. Additional Measures Requested Of The FDA:

A. The FDA should designate as *scientifically satisfactory* any and all methods validated as acceptable alternatives to one or more animal tests currently in use, as evidenced by approval and enactment by any of the participant members of ICH (the US, the EU, and Japan), or by the FDA itself.

B. The FDA should develop and implement standardized procedures requiring that its drug and device application reviewers accept as valid and sufficient any and all data submitted using scientifically satisfactory alternatives to animal test methods. Individual FDA reviewers must not require additional animal test data in such instances, or applicants will seek to include such data in initial applications so as to avoid approval delays.

C. The FDA should use the strongest possible language in its industry guidances regarding the acceptability and sufficiency of scientifically satisfactory non-animal alternatives, clearly indicating that the FDA requires only the non-animal data and does not require (and will not request) any animal test data thereby replaced.

D. The FDA should support efforts to obtain adequate funding directed specifically toward the development and utilization of its extensive human drug database as a particularly effective method for improving preclinical and clinical drug testing and public safety, informing FDA decisions regarding content of new drug or device applications, and replacing animal test methods with scientifically satisfactory non-animal alternative methods.

B. STATEMENT OF GROUNDS

I. Introduction

I.1 Purpose

This petition is submitted to the FDA, and seeks changes in FDA regulations and policies that would require, and not merely permit or recommend, that pharmaceutical companies, device manufacturers, and other entities regulated by the FDA submit data only from scientifically satisfactory non-animal alternatives in lieu of corresponding animal test methods, whenever such scientifically satisfactory alternatives are available, when carrying out the requirements of the Federal Food, Drug and Cosmetic Act (FDCA), 21 U.S.C. §§ 351, 355.

I.2 Rationale

There are sound scientific, economic and humane reasons why the use of valid non-animal methods should be mandatory. Yet currently the United States has no federal requirement for the use of non-animal methods to replace animals in research, testing or education. The National Institutes of Health (NIH) currently recommends that non-animal methods be considered for research funded by that agency (see NIH *Guide for the Care and Use of Laboratory Animals*). However, even if readily available, such methods are not mandatory. Additionally, ICCVAM has validated few non-animal methods, and

ICCVAM's member agencies are not required to adopt any of its recommendations. Thus, there is little effective incentive or support for researchers, regulators and educators to adopt available non-animal test methods, or to develop new ones.

In contrast, Europe has progressed with the development, promotion and implementation of non-animal methods following the passage in 1986 of EU Directive 86/609/EEC. This legislation gives impetus to the goal of minimizing animals in experiments by requiring – not merely recommending – the use of alternatives. In particular, Article 7.2 states, "An experiment shall not be performed [on an animal], if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available." This requirement is legally binding on all member states of the EU. Adoption of a similar regulation in the US would go far toward harmonizing policy and practice in the US and the EU.

The scientific, economic, and humane benefits of non-animal methods are no longer in doubt (Balls et al., 2000, 2004). And while the validation and regulatory acceptance of alternative methods have proceeded more slowly than some would have predicted or hoped, there now exist several effective alternatives to animal test methods that have crossed these important thresholds. Yet in the US there is no corresponding regulatory requirement to use such methods.

It is appropriate to change FDA regulations and practices to require and promote the use of such alternatives. Such changes would recognize the importance of developing and adopting non-animal methods to improve preclinical drug and device development, improve the safety and effectiveness of drugs and devices approved for human use, support efforts to harmonize international regulations and practices, and greatly advance animal welfare.

I.3 Scope of the Petition

While the principle of mandating scientifically satisfactory replacement methods applies to research and education as well as to drug and device testing, it is in the latter realm that validation and regulatory acceptance of non-animal methods is best defined. However, some consideration of these other areas, and of the replacement of animal-based methods in education in particular, is revealing. For example, some 30 empirical studies have so far been published showing equal or greater efficacy for non-animal methods (Balcombe, 2001; De Boo and Knight, 2004). **Appendix A** presents brief overviews of these areas.

I.4 FDA Authority to Take the Requested Action

The FDA has jurisdiction under the FDCA[1] to issue regulations mandating the use of scientifically validated non-animal methods in lieu of animal test methods whenever such validated alternatives are available and would be equal or more effective predictors of the drugs or devices for which approval is sought.[2]

1 21 U.S.C. §§ 301–399.
2 *See* 21 U.S.C. § 371.

Absent an unambiguously expressed intent by Congress, administrative agencies are afforded great deference in their interpretation of the statutes that they enforce.[3] An agency's exercise of regulatory authority may be overturned only if it is arbitrary and capricious, an abuse of discretion, or not in accordance with the law.[4] Thus, the validity of a regulation promulgated under the FDCA is presumed so long as it is "reasonably related to the purposes of the enabling legislation."[5]

The language of the FDCA is unambiguous in granting the authority to regulate both drugs and devices.[6] Such authority extends to the manner in which those drugs and devices are tested prior to their submission for FDA approval. The FDCA expressly provides that the FDA has "the authority to promulgate regulations for the efficient enforcement of the Act."[7] Because the primary objective of the FDCA is the protection of public health,[8] its rulemaking authority must be construed broadly. As one federal court has stated,

> The primary objective of the Federal Food, Drug and Cosmetic Act is the protection of the public health. As such, [FDA's] rulemaking authority under Section 701(a) has been broadly construed to uphold a wide variety of assertions of regulatory power. . . . We read (Section 701(a)) as analogous to the provision "make . . . such rules and regulations as may be necessary to carry out the provisions of this Act," in which case "the validity of a regulation promulgated thereunder will be sustained so long as it is 'reasonably related to the purposes of the enabling legislation.'" When agency rulemaking serves the purposes of the statute, courts should refuse to adopt a narrow construction of the enabling legislation which would undercut the agency's authority to promulgate such rules.[9]

Indeed, in the 1980s the FDA promulgated regulations regarding animal testing pursuant to the FDCA,[10] demonstrating that it construes the FDCA to grant it broad authority to mandate the kind of testing that will be required to meet the relevant statutory standards.

Moreover, Congress has expressly authorized the FDA to regulate animal testing in drug and device development by permanently establishing ICCVAM, an interagency committee composed of representatives from 15 federal regulatory and research agencies, including FDA,[11] that use, generate, or disseminate toxicological and safety information. Among the purposes of ICCVAM are to "increase the efficiency and effectiveness of Federal agency test method review" and to reduce and replace

---

3 *Chevron U.S.A. Inc. v. NRDC, Inc.*, 467 U.S. 837, 842–43 (1984).
4 *See* 5 U.S.C. § 706(2)(A).
5 *See Mourning v. Family Publications Inc.*, 411 U.S. 356, 369 (1973).
6 *See* 21 U.S.C. § 321(g)–(h).
7 21 U.S.C. § 371(a).
8 *An Article of Drug*, 394 U.S. at 798.
9 *Pharmaceutical Mfrs. Ass'n*, 484 F. Supp. at 1183.
10 *See* 21 C.F.R. §§ 312.23(a), 312.88, 314.50(d).
11 42 U.S.C. § 285l-3(c)(9).

animals in testing, when feasible.[12] Final decisions regarding reduction and replacement of animal testing are left to each individual agency's discretion.[13] Accordingly, the FDA has ample authority to implement the actions requested by this petition.

I.5 Humane Problems

A federal agency has a duty to the public that supplies the money for the research it funds. Accordingly, when an independent poll shows that 75 percent of Americans disapprove (57 percent strongly disapprove) of all animal research and testing that cause severe pain and distress, and that fully one-third of the American population objects to all animal experimentation whatever the reason or level of pain and distress (HSUS 2001), the FDA is obligated to address these concerns. These sentiments are confirmed by other polls. In May 2006, almost one million people expressed their opinion on this issue via the Sky News website (www.sky.com/skynews). More than 51 percent answered "No" to the simple question, "Are you in favor of animal testing?"

Animal testing inherently and unavoidably causes animal pain, distress, and suffering. In many cases the suffering may be especially severe and prolonged, such as in tests for acute and chronic toxicity, carcinogenicity, skin irritancy and corrosivity, as well as the Draize eye irritancy tests. According to protocols established by the National Toxicology Program, animals used in chronic toxicity and carcinogenicity studies receive the test substance daily, seven days a week, for two years with no recovery periods (NTP, 2006). Data from the National Institute of Environmental Health Sciences (NIEHS) on the two-year survival rate for rats undergoing chronic toxicity studies indicate that between 25 and 70 percent of animals die before the end of the two-year study (Haseman, 2003).

The Organisation for Economic Co-operation and Development (OECD) has published guidelines for assessing clinical signs for animals used in toxicity testing (OECD, 2000). It lists the following as some of the common conditions and clinical signs that may occur during toxicity testing that indicate an animal is experiencing pain and/or distress: Gasping, difficulty breathing, tremor, seizures, abnormal vocalization, diarrhea, vomiting, bleeding from any orifice, edema, abdominal rigidity, rectal or vaginal prolapse, swollen joints, and paralysis.

In toxicity testing, pain- or distress-relieving drugs are typically withheld for a variety of reasons, including the concern that they might alter the toxicity profile of the chemical being tested (Stephens, 2000). Overall, acute and chronic toxicity testing is rightfully regarded as imposing moderate to severe pain on experimental animals (Combes, 2002; NRC, 1992). At least two regulatory entities, the OECD and the Australian government, have banned the use of the especially cruel LD50 acute toxicity test (OECD, 2001; Australian Government, 1993).

Significant pain and distress arising from orogastric gavage, a procedure used extensively on animals in toxicologic and pharmacologic studies, has been documented. Gavage is typically used daily to administer the test dose directly into the stomach of an experimental animal. It involves forcible restraint followed by insertion of a tube into the

[12] 42 U.S.C. § 285l-3(b).
[13] 42 U.S.C. § 285l-3(e).

mouth, then through the esophagus into the stomach.

Complications from this procedure are numerous and significant, and include accidental administration of test substances into the trachea and lungs, aspiration pneumonia, esophageal trauma or perforation, gastric distension, edematous lungs, hemothorax and death (Balcombe, 2004). One study compared a group of rats who were sham gavaged for 10 days with a dry needle with another group who were dosed using gavage for 10 days with a test substance (cyproterone acetate). After the 10 day trial, both groups exhibited massive hepatic disease, suggesting that the gavage procedure alone, and not the test chemical, was responsible for the resulting pathology (Roberts, 1995).

Data from the United States and Canada indicate that testing procedures account for the vast majority of animals reported in the highest categories of pain and distress (Stephens et al., 2002). The USDA's Annual Report of Enforcement for 2004 documented that 86,748 animals were classified under Category E – experiencing pain or distress without relief from drugs. This number does not include species not covered by the AWA – rats, mice, birds, and fish – who account for the great majority of animals used in testing. These findings underscore the importance of replacing animal use in regulatory toxicity testing.

Overall it appears that the US under-reports pain and distress associated with testing. For example, Canada reports that approximately 62 percent of the animals reported as experiencing moderate to severe pain and distress were used in testing, in comparison to 7.4 percent reported by the US. The drastically lower US statistics in both broad areas of animal use cannot be explained entirely by the omission of mouse, rat, bird, and fish data in the US figures.

Animal pain and distress are also directly impacted by violations of the Animal Welfare Act (AWA; 7 U.S.C. §2131-§2156). According to figures published by the USDA, the AWA was violated a total of 20,845 times during the reporting year ending in September 2005, affecting a total of 1,364,358 animals covered by the Act. This is an increase of 2,570 violations (14 percent) and 981,535 (256 percent) animals from the previous year, representing a three-year increase of 44 percent in violations (6,384) and 321 percent in animals affected (1,040,268) since 2002.

The true statistics may well be considerably higher. The USDA's Office of the Inspector General has reported that the Animal Care Unit's Eastern Region does not aggressively pursue enforcement actions against violators of the AWA, and that because facilities are realizing there is no consequence for violating the AWA, the number of repeat violators in the Eastern Region is increasing (APHIS, 2005). Furthermore, The Humane Society of the United States analyzed a year of reports submitted by the top 50 NIH-funded US research institutions and uncovered what they judge to be many experiments that caused pain and distress to animals, but were apparently not reported to the USDA (HSUS, undated). Because resources are limited for monitoring the large number of US animal laboratories, it is likely that many cases of noncompliance with animal welfare laws and guidelines go unreported.

Other factors resulting in the inherent suffering of animals used in research include the manner in which animals are housed, transported, handled, and used. Numerous examples exist of non-human primates used in research dying unnecessarily due to infections,

neurological and heart problems, and failures of heating/ventilation systems in their living quarters or transport crates.

For decades, hundreds of thousands of dogs and cats have been supplied to laboratories from Class B animal dealers, including random source animals such as pets, strays and animals in pounds and shelters. Class B random source dealers have been shown to keep animals in crowded and unsanitary conditions, with little or no veterinary attention, poor food and insufficient water. Some animals fail to survive their time with dealers, and exposés such as the critically acclaimed 2006 HBO documentary *Dealing Dogs* have dramatized some of the worst offenders. This is a major contributory factor to animal suffering even before these animals become experimental subjects. In 2001 there were over 32,000 dogs and cats from Class B dealers in American laboratories, and in 2004 they numbered approximately 18,500 (HSUS, 2006).

Notwithstanding the problems of under-reporting of pain and distress, deaths associated with animal housing, transport and usage, and the nature of many experiments themselves, stress and distress arising from everyday laboratory living conditions create an even greater incentive for replacing the use of animals in laboratories.

A recent literature review reported on the effects of common laboratory routines, including handling, moving or cleaning cages, blood collection, and orogastric gavage, on physiological markers of stress. The authors related that animals (rats, mice, rabbits, hamsters, monkeys and various bird species) exhibited rapid, pronounced, and statistically significant elevations of physiological stress indicators such as heart rate, blood pressure and a variety of hormone levels in response to these daily perturbations. The elevations typically ranged from 20 to 100 percent or more above baseline, usually lasting 30 to 60 minutes or longer. The data suggest that significant fear, stress and possibly distress are predictable consequences of standard laboratory procedures, and that these phenomena not only cause animal suffering, but also may distort physiological measures and scientific outcomes (Balcombe et al., 2004).

This tendency for stress is compounded by standard laboratory housing conditions, which impose unnatural levels of confinement and commonly deprive the occupants of opportunities to engage in essential natural behaviors, including exploring, foraging, nesting, hiding, and in many cases social interaction with other members of their own species (Olsson and Dahlborn, 2002; Hurst et al., 1999).

The link between abnormal or impoverished housing conditions and the development of behavioral stereotypies is also well established. Cage stereotypies – repetitive, unvarying and apparently functionless behavior patterns such as rocking, bar-gnawing, digging, pacing, head-banging and ingesting feces – are commonly seen in animals kept in close confinement. They are believed to reflect animal suffering (Mason, 1991) and are common in some rodents caged for research, including mice, chinchillas, black rats, deer mice, field voles, bank voles, and gerbils (Garner and Mason, 2002). Stereotypies are estimated to afflict some 50 percent of all laboratory-housed mice (Mason & Latham, 2004). Other manifestations of distress in laboratory environments include self-mutilation and various neuroses, such as trichotillomania (compulsive hair-pulling) in primates, barbering (whisker removal) in rodents, and polydipsia (excessive drinking) in rabbits.

The impact of typical procedures on individual animals gives more meaning to the numbers and generalizations cited above, and provides a clearer picture of the scale of distress and suffering. For example, "knockdowns" are routine for chimpanzees used in HIV, hepatitis, and pharmaceutical testing. Because chimpanzees are much stronger than humans, they often must be anesthetized for even minor procedures such as drawing blood or injections. A knockdown typically involves one or more workers approaching the chimpanzee with a dart gun loaded with anesthetic; often, several darts are required and chimpanzees scream and thrash around to avoid them. Darts may hit them in the face or other sensitive areas, and many animals lose bladder and bowel control during these procedures. Chimpanzees also know when a knockdown is about to occur because their food and water is withheld – leaving them in stressful anticipation.

Subsequent liver biopsies are frequently performed, which are invasive and painful procedures that add greatly to the ordeal for individual animals. For example, in 14 years at a US laboratory one chimpanzee involved in HIV experiments was knocked down over 289 times, and endured some 40 punch liver biopsies, three open wedge liver biopsies, three bone marrow biopsies and two lymph node biopsies. He chewed off his thumbs waking up alone from knockdowns when no one was around to care for him, and during one fit of anxiety bit off his index finger. Anxiety attacks since his release to a sanctuary were so bad he was often found choking, gagging and convulsing (Fauna Foundation *a*).

Three other chimpanzees involved in hepatitis B experiments are estimated, based on reported procedures, to have been subjected to 29 dart knockdowns each during the six and a half months' duration of a particular investigation (Wieland et al. 2004). According to Fauna Foundation, "It can take up to five darts to put an adult chimp down. Some biomedical chimpanzees have been knocked down up to 220 times and have had over 130 liver biopsies done." (Fauna Foundation *b*)

The examples in this section demonstrate that pain and distress are widespread and considerable for animals in laboratories, and lend compelling weight to arguments for replacing their use.

II. History of Replacement Methods for Animal Use

Since the publication in 1959 of *The Principles of Humane Experimental Technique* (Russell and Burch, 1959), the development of replacement methods for animal use in testing, research and education has burgeoned. During that time, the "Three Rs" concept of *replacement, reduction,* and *refinement* has been codified into laws, policies and guidelines throughout the world, and has become the focus of several government and academic centers. The adoption of non-animal methods has become almost universally accepted as a laudable goal. And while the pace of their adoption has lagged far behind their development, validation and regulatory acceptance, legislation mandating their use (**Subsection B.IV,** below), is now being realized.

A number of important historical events have helped bring non-animal methods increasingly onto the US agenda. The AWA, first enacted in 1966 and amended several times since, codifies Congress' determination that animal welfare concerns must be afforded a high priority. The 1985 amendments to the AWA required that every facility conducting research on animals have an Institutional Animal Care and Use Committee (IACUC), charged with reviewing proposed animal use protocols in the context of animal

welfare, and ensuring that investigators have considered the use of 3Rs principles. The US Public Health Service's *Guide for the Care and Use of Laboratory Animals*, now in its seventh edition, sets out minimum standards of animal care and husbandry (NRC, 1996), by which facilities conducting animal research funded by the NIH must also abide.

A brief chronology of significant events addressing alternatives to animal use illustrates the growing attention that has been given to non-animal methods in the US during the past quarter century:

- 1981: Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is founded
- 1993: The NIH Revitalization Act requires NIH to develop and promote alternative methods for toxicity testing and other research involving animals
- 1993: First World Congress on Alternatives and Animal Use in the Life Sciences is held in Baltimore
- 1997: ICCVAM is formed to increase validation and implementation of alternatives
- 1997: USDA APHIS Animal Care Policy Manual adds Policy #12 (Consideration of Alternatives to Painful/Distressful Procedures), requiring investigators to consider using alternatives to painful and distressful procedures
- 1999: ICCVAM approves Corrositex™ non-animal test for skin corrosivity
- 2000: USDA issues revised Policy #12, requiring investigators to provide written narratives justifying animal use rather than alternative methods
- 2002: Fourth World Congress on Alternatives and Animal Use in the Life Sciences is held in New Orleans
- 2002: OECD approves several non-animal test methods, including EpiSkin™, EpiDerm™, and in vitro tests for percutaneous absorption
- 2002: LD50 test is de-listed from international guidelines governing chemicals testing

III. Current Practice

III.1 Alternative Methods

III.1.1 Advantages of Non-Animal Methods

The potential benefits of replacing animal methods are manifold. Non-animal methods, which include epidemiological and clinical studies, in vitro methods, computer modeling and simulation, human tissue studies, microfluidics methods, microdosing and other approaches have more predictive value and specificity to the human condition than do animal methods, which rely on different species with different anatomies and physiologies. Non-animal methods also have the inherent ethical advantage of sparing significant numbers of animals from the pain and distress commonly associated with laboratory use, a goal consistent with most public opinion polls. Americans, even when they support animal research, do not want to see animals suffer. And non-animal methods are often less costly and less time-consuming to perform, and may require lower investment in personnel than animal-based methods (**Subsection B.*III.2.3*** below).

In addition to the methods listed in **Subsection B.*III.1.2*** below, there are long-standing validated non-animal testing methods already in use. The Multicentre Evaluation of In-Vitro Cytotoxicity (MEIC) method was validated in a trial based in Uppsala, Sweden

(MEIC, undated). It tested a set of 50 chemicals for which there were both a well-defined human toxicological profile and substantial data on acute toxicity in animals. Using 61 different in vitro assays, scientists from 29 independent laboratories demonstrated that these human cell line tests were more predictive of human toxicity than animal data. While animal tests were at best only about 65 percent predictive of human acute toxicity, a combination of four cell tests was able to determine if a substance is dangerous to humans with better than 80 percent precision.

The MEIC battery has been proclaimed "well suited for comparisons with the most common criteria of human and animal toxicity (average human blood concentrations, LD50 values, etc.)" (Clemedson et al., 1996; Ekwall et al., 1998). These encouraging results prompted the initiation of the EDIT-project (Evaluation-guided Development of *In-vitro* Test batteries) (Ekwall et al., 1999), which served to optimize the original MEIC test battery by establishing and validating new in vitro tests relevant to biokinetics and for organ-specific toxicity. Based on the success of these programs, the ACuteTox project is currently underway to improve the prediction of human systemic toxicity still further, by identifying factors that can eliminate misclassifications and introducing additional parameters such as absorption, distribution, elimination, metabolism and organ specificity (Clemedson et al., 2005).

The US National Cancer Institute (NCI) drug discovery and development arm (the Developmental Therapeutics Program [DTP]) has developed and implemented superior non-animal testing methods for carcinogenicity, anti-HIV drug efficacy, and certain categories of cell toxicity. A panel of 59 human tumor cell lines (DTP Human Tumor Cell Line Screen) is used to identify compounds with anti-tumor effects (DTP*a*, undated), and a second panel of about 100 human cell lines is used to test compounds for cytotoxicity (Kerkvliet, 1990). NCI developed these methodologies in the late 1980s because of its dissatisfaction with the poor predictability of animal testing in these areas. The DTP AIDS antiviral screen uses human HIV-1 cell lines to identify compounds with anti-HIV activity (DTP*b*, undated). Wider applications of these superior non-animal methods seem justified.

Microdosing ("phase 0" clinical trial) is a relatively recent development that shows great promise to revolutionize drug development and predictive drug toxicology. By administering a very small sub-pharmacological dose of a potential new drug to human volunteers, important and predictive human-specific data can be obtained, with no need for cross-species extrapolation and allometric scaling, both of which are demonstrably poor. Doses approximate one-hundredth of the pharmacological dose (that can be derived from in vitro models), with a maximum administered dose of 100μg (EMEA, 2003).

By sampling the subject's body fluids (such as urine, blood and sweat) and analyzing them by accelerator mass spectrometry, valuable human-specific pharmacokinetic and pharmacodynamic properties of the potential new drug can be determined in conjunction with complementary technologies such as positron emission tomography. In combination with in silico data analysis and modeling, this information can greatly aid decisions to proceed to later stage clinical trials that determine human efficacy and toxicity.

Although some proponents of microdosing support its use in concert with traditional animal studies, others argue that it enables, along with comprehensive data from in vitro human-specific studies, a safer and more reliable approach to drug development that is

devoid of any requirement for animal testing. All informed parties agree that microdosing provides a means to greatly reduce the number of animals used in the process.

In February 2005, results from the Consortium for Resourcing and Evaluating AMS Microdosing (CREAM) trial revealed that concerns about the accuracy and applicability of microdosing are overstated. A panel of drugs was selected for their difficult pharmacokinetic properties to strongly challenge the method. Three of the five selected drugs resulted in predictive human pharmacokinetic data, which would have allowed the right decision to be made for further drug development. And though the other two deviated from linear pharmacokinetic behavior, the results gave very useful insights into the properties of the drugs (CREAM, 2005; Lappin et al., 2006). The stakeholders are currently involved in a collaborative project to test seven pharmaceuticals (EUMAPP; the EU Microdose AMS Partnership Programme) for the reliability of microdosing to predict pharmacokinetic properties at pharmaceutical doses, and also to improve in silico modeling applications to analyze the data produced.

Currently, phase I and II clinical trials of pharmaceuticals are the first steps in the drug development process that actually address human responses. Phase I trials use small and gradually increasing doses of the candidate drug to assess absorption, distribution, metabolism, excretion, and toxicity (ADMET) in a small population of human volunteers (often 20-80 subjects). Approximately 40 percent of drugs fail in phase I trials due to toxicities or inappropriate pharmacokinetic properties not identified by the preceding animal testing phase (Dimasi, 2001). The remaining 60 percent of candidate drugs progress to phase II trials, which usually involve 100-500 patients and provide a broader look at short-term side effects and toxicities. Both types of trial commonly refute animal data regarding ADMET, side effects, and efficacy, as is discussed in greater detail below (**Subsection B.*III.2.2***).

It is widely accepted that all major pharmaceutical companies are now using "-omics" technologies to varying degrees in their drug discovery and development pipelines. A large number of companies specializing in in vitro methods offer an ever-expanding suite of assays and screens to the pharmaceutical industry, including focused toxicogenomic assays using multiple human cell types (that can be co-cultured for maximum physiological relevance) and microarray chips designed to identify limited but highly specific subsets of human toxicity-responsive genes.

When used in conjunction with rapidly advancing, powerful and complementary proteomic and metabonomic technologies that allow elucidation of the molecular basis of toxic response and disease states at the protein and metabolite levels as well as genetically, then the value of these approaches becomes greater than the sum of the parts. They have been used in hundreds of successful experiments to date; for example to classify diverse types of blinded compounds by chemical class and mechanism (Hughes et al., 2000; Scherf et al., 2000; Steiner et al., 2004; Thomas et al., 2001), to classify human tumors by origin and type (Chung et al., 2002), to derive prognoses for breast and ovarian cancer patients, to select breast cancer patients for appropriate follow-up chemotherapy (Rai et al., 2002; Chang et al., 2003; van't Veer et al., 2002), and to obtain human efficacy and tolerability data for new drugs (Rappsberger et al., 2002).

As exciting as they are, "-omics"' technologies are just a small fraction of the plethora of non-animal techniques helping industry in drug development and the testing of new

pharmaceuticals and chemical entities. Researchers are advancing rapidly in their understanding of human disease and responses to drugs and toxins, devoid of interspecies extrapolation problems. For example, the British company Pharmagene, dedicated to drug development using human-specific methods, merged with Asterand in 2006 and now offers a host of in vitro methods to ascertain the pharmacokinetic, pharmacodynamic and ADMET properties of new drugs. Several drugs recently developed and tested using in vitro methods (for example, for pain relief and for cystic fibrosis), are currently producing positive data in human clinical trials.[14]

There are many more such biotechnology companies. Stem Cell Innovations (formerly Amphioxus) has created a stable human liver cell line called *ACTIVETox* that reproduces all human normal liver functions, allowing human-specific high throughput screening for ADMET properties in drug development and detailed metabolic studies. This showed excellent correlation with calculated human LD50 values, for example, from 26 compounds used in the aforementioned MEIC toxicity study. GenPharmTox of Germany offers a comprehensive range of recombinant cell lines expressing human CYP enzymes, and a variety of phase II enzymes, relevant in the metabolism of xenobiotics.

These systems avoid the problems caused by interspecies heterogeneity, particularly regarding ADMET, and provide a higher predictivity due to their human context. The company CEREP has developed the *Bioprint* database that collates information from over 140 well-characterized in vitro assays on over 2000 currently marketed drugs, withdrawn products, failed development candidates and reference compounds, to allow a deeper understanding of the relationships between these properties and 200 reported adverse drug reactions (ADRs), and allow the prediction of human in vivo responses.

Beyond the safety testing and drug development arenas, non-animal methods of research have contributed far more to our understanding of *human* health and medicine than animal experiments ever can. Epidemiological studies of human populations showed the links between tobacco and cancer, between cholesterol and heart disease, between high-fat diets and common cancers, and between high blood pressure and stroke. They revealed how the AIDS virus is transmitted, and how to prevent the disease. Human clinical research showed that pancreatic damage was the cause of diabetes long before Banting and Best performed their well-known dog experiments. And the authors of an editorial in *Stroke* concluded that "the answers to many of our questions regarding the underlying pathophysiology and treatment of stroke do not lie with continued attempts to model the human situation perfectly in animals, but rather with the development of techniques to enable the study of more basic metabolism, pathophysiology, and anatomical imaging detail in living humans" (Wiebers et al., 1990).

The causes of disease are also often elucidated in the course of treating patients. Sophisticated scanning technologies (CT, PET and MRI) have isolated abnormalities in the brains of patients suffering from Alzheimer's disease, schizophrenia, epilepsy, and autism. Dietary studies of multiple sclerosis have shown how a low-fat diet significantly reduces morbidity and mortality from this disease. Autopsy studies have revealed that Alzheimer's disease patients have abnormally high aluminum concentrations in their

[14] These examples were developed by Pharmagene (UK), and are now being further developed by other companies since their merger with Asterand (US).

brains, and that characteristic brainstem abnormalities occur in babies with sudden infant death syndrome.

The UK-based Dr Hadwen Trust (www.drhadwentrust.org.uk) has been instrumental in the movement toward more effective research and testing regimens by funding numerous and varied projects for decades. Their programs have been immensely successful not only in replacing the use of animals in many areas, but also in developing more pertinent data to serve human health and safety. Their research was pivotal to the replacement in the UK and elsewhere of the Draize eye test for severe eye irritants. In cancer therapy, it enabled better assessment of cancer drugs and improved cancer treatments without animals, including the development of a method utilizing patients' own tissues to determine the most effective personalized treatments. The Trust's sponsored research also replaced animals used in routine botulinum final batch testing in the UK via a better in vitro alternative; development of a cell culture method to aid the discovery of human-specific drugs to attack tumors via their blood supply; and capillary electrophoresis to aid drug selection without animal use, especially in anti-malaria and anti-cancer programs.

The list of examples continues, from sources other than the Dr Hadwen Trust:

- A tissue culture test for diagnosing rabies has replaced methods that involved infecting mice, shortening diagnosis time from 35 days to just four days and saving 30,000 animals annually.
- In monoclonal antibody production, cell culture methods are now replacing the induction of painful abdominal tumors in mice and rats. In the UK for example, use of animals for monoclonal antibody production has dropped by 87 percent since 1990 when more than 46,000 animals were used each year.
- Pregnancy testing once involved injecting urine samples into rabbits, who were killed several days later and their ovaries examined. Pregnancy kits now utilize a fast and simple test tube method, which relies on monoclonal antibodies produced by cell cultures (see above).
- The production and testing of polio vaccine is now done mostly in vitro in accordance with a recommendation by the World Health Organization. This used to require around 55,000 monkeys per year. This in vitro test known as MAPREC (Mutant Analysis by Polymerase chain reaction and Restriction Enzyme Cleavage) is considered by the US Center for Biologics Evaluation and Research to be more sensitive than monkey tests, which have failed to detect vaccine batches with deliberately induced mutations.

Although this petition concentrates on animal *testing* (in line with the responsibilities of the FDA), some attention to the establishment, development and implementation of replacement methods in the arenas of biomedical research and education can be enormously informative for this particular application. Examples from research and education can be found in **Appendix A**.

III.1.2 Validation

Animal tests, many of which have been in use for decades, have never been scientifically validated (assessed in multiple laboratories to see if they provide consistent predictive data with specific application to humans) (Stephens, 2000). However, ECVAM is currently exploring an initiative to *invalidate* inappropriate testing methods (Balls and

Combes, 2005), and has recently stressed the urgent need for critical self-appraisal in toxicology and a critical analysis of toxicology tools, to give each its rightful place in current and future testing strategies (Hoffmann and Hartung, 2006). Despite this, a bias persists towards animal methods. A wealth of anecdotal evidence indicates that regulatory bodies feel more comfortable with animal test data than with data from non-animal tests, even when those animal tests are known to be unreliable and of questionable relevance (Balls, 2004; O'Connor, 1997).

The validation of non-animal methods is a key step in their eventual implementation. For the purposes of this discussion, a valid method (animal or non-animal) is one that is "scientifically established as relevant and reliable for a particular purpose" (Balls, 2004). Ideally, comparisons of non-animal methods with more traditional animal methods should be made by seeing how well each compares to an independent reliable standard. In principle, that standard should be human data, but such data are often lacking due to ethical limitations on human testing. And while some human clinical data exist from occupational or accidental exposures and from suicides, they are often deemed unreliable or of limited precision and availability (Stephens, 2000).

Unfortunately, with no comprehensive human data standards available for many applications, the animal test itself has typically been used as the default standard against which a non-animal test is measured. This approach is limited by the inherent problem of fluctuations in animal data as well as the many reasons such data have poor applicability to humans, and thus distorts comparisons to favor animal test results. For example, the coefficient of variation (standard deviation divided by the mean) in eye irritancy testing studies using animals has been shown to be approximately 0.5 (the range is 0 to 1.0). By contrast, in vitro tests have a much lower coefficient of variation of around 0.2. Yet, when one compares the in vitro tests to the animal tests, using the latter as the standard of measure, the in vitro data show only a 70-80 percent correlation with the animal data, due to the latter's natural variance (Bruner et al., 1996). It is difficult to hit a moving target.

For this reason, most of the more promising non-animal tests demonstrate no more than a 70 percent correlation with pre-existing animal tests, and 90 percent correlation is around the absolute upper limit. Thus, non-animal methods *seem* inferior to animal tests even when they may be substantially superior, ironically due to a shortcoming (biological variability) in the animal paradigm (Stephens, 2000).

Notwithstanding these various factors working against their acceptance, a number of animal replacement and reduction methods have emerged favorably from the scientific validation process. As of August 2007, ECVAM lists 27 such methods that have been scientifically validated:

1) EpiDerm™ skin corrosivity test (March 1998)
2) Rat Transcutaneous Electrical Resistance (TER) skin corrosivity test (April 1998)
3) Episkin® skin corrosivity test (April 1998)
4) In vitro production of monoclonal antibodies (May 1998)
5) 3T3 Neutral Red Uptake (NRU) phototoxicity test (May 1998)
6) Local Lymph Node Assay (LLNA) for skin sensitization (March 1999)
7) Toxin Binding Inhibition (ToBI) test for batch potency testing of tetanus vaccines for human use (December 2000)

8) ELISA test for batch potency testing of tetanus vaccines for human use (December 2000)
9) Corrositex™ assay for skin corrosivity (December 2000)
10) Whole rat embryo embryotoxicity test (May 2002)
11) Micromass embryotoxicity assay (May 2002)
12) Embryonic stem cell test for embryotoxicity (May 2002)
13) ELISA test for batch potency testing of erysipelas vaccines (June 2002)
14) Colony Forming Unit-Granulocyte/Macrophage (CFU-GM) assay for predicting acute neutropenia in humans (March 2006)
15) Upper Threshold Concentration (UTC) step-down strategy in acute aquatic toxicity testing (March 2006)
16) Human Whole Blood IL-1 in vitro pyrogen test (March 2006)
17) Human Whole Blood IL-6 in vitro pyrogen test (March 2006)
18) PBMC IL-6 in vitro pyrogen test (March 2006)
19) MM6 IL-6 in vitro pyrogen test (March 2006)
20) Human Cryopreserved Whole Blood IL-1 in vitro pyrogen test (March 2006)
21) SkinEthic™ Human Skin Model for skin corrosivity testing (November 2006)
22) Micronucleus test for genotoxicity testing (November 2006)
23) Bovine Corneal Opacity and Permeability (BCOP) test for ocular corrosivity and severe eye irritants (April 2007)
24) Isolated Chicken Eye (ICE) test for ocular corrosivity and severe eye irritants (April 2007)
25) Reduced Local Lymph Node Assay (rLLNA) for skin sensitization (April 2007)
26) Episkin® artificial skin model for skin irritation (April 2007)
27) EpiDerm™ artificial skin model for skin irritation (April 2007)

A few details regarding a small sample of these methods illustrate the validation process:

Skin Corrosivity: EpiDerm™ and Episkin®

The EpiDerm™ test, which uses a reconstituted three-dimensional human epidermis model, showed excellent prediction of corrosivity for a wide spectrum of chemicals in prevalidation studies (Liebsch et al., 1999). A full-scale validation study was completed by ECVAM in 1996 and 1997, involving a total of 60 chemical compounds. It has been approved as a replacement for live animal skin corrosivity tests for hazard identification and classification of corrosive potential, and fulfills international regulatory requirements for handling, packing and transport of chemicals. EpiDerm™ and its companion method Episkin®, (also listed above) are now included in EU Directive 67/548/EEC for the Classification, Packaging and Labeling of Dangerous Substances, and have been granted acceptance by OECD.

Corrositex™

This skin corrosivity test uses an artificial skin barrier made of collagen on which a chemical or chemical mixture is applied. The test substance is then assessed for latency of chemical penetration and resulting color change. This method was accepted as valid by ICCVAM in June 1999, and the US Department of Transportation (US DOT) accepts the use of Corrositex™ for labeling of potentially dangerous goods (Huggins, 2003a). Because of ICCVAM's government-based membership, a positive recommendation by its

peer review committee is considered tantamount to federal government approval (Stephens, 2000).

Embryonic Stem Cell Test

This test, which uses a propagated cell line, is a replacement for traditional animal methods requiring at least 80-160 animals per assay (Huggins, 2003b). In 2001, the ECVAM Scientific Advisory Committee (ESAC) unanimously declared it to be highly reproducible, to have good correlation between in vitro and in vivo data, and to be applicable for testing a diverse group of potentially embryotoxic chemicals. These conclusions followed the successful outcomes of prevalidation and validation studies conducted between 1996 and 2000. Thus the EST was deemed by ECVAM as ready to be considered for regulatory purposes (Genschow et al., 2002).

III.1.3 Regulatory Approval

Perhaps the greatest obstacle to the replacement of animal methods with non-animal methods is that national and international laws, regulations and guidelines tend either to encourage or require the animal method. Until the last decade there was no formal process for regulatory agencies in the US to evaluate non-animal methods for acceptance. This state of affairs spawned the formation of ICCVAM in 1997. ICCVAM comprises representatives from 15 federal agencies. With the subsequent establishment of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in 1998, ICCVAM began reviewing alternative methods for regulatory acceptability.

Unfortunately, the process of regulatory approval of alternative methods in the US has been slow. To date, the only methods validated by ICCVAM are Episkin®, EpiDerm™, Corrositex™, and the Rat TER assay for assessing dermal corrosion; the local lymph node assay (LLNA) for assessing the allergic contact dermatitis potential of chemicals; and the up-and-down (UDP) procedure for acute oral toxicity (ICCVAM, 2005). Despite this, many cosmetics and personal products companies have voluntarily stopped testing on animals altogether, and many more have always shunned animal testing. The CCIC (Coalition for Consumer Information on Cosmetics) currently lists 180 companies that do not use animal testing in the development or manufacture of their products (CCIC, 2005).

And while proprietary concerns keep most companies from divulging the results of their tests, there is now widespread use of replacement methods in industry. In the case of ocular irritation tests, for example, many individual companies are using diverse in vitro ocular irritation tests (e.g. the Hen's Egg Test-Chorioallantoic Membrane [HET-CAM] assay, the Chorioallantoic Membrane Vascular [CAMVA] assay, and MatTek's EpiOcular™ (http://www.mattek.com/pages/products/epiocular, the only completely non-animal method) to gain important safety and efficacy information about their products and raw materials, eliminating the need for animal testing in the process (Curren and Harbell, 2002). EpiOcular™ is used by multinational companies such as Avon, Colgate-Palmolive, and Procter & Gamble due to its use of human-derived cells, excellent correlation with in vivo results, and high level of long-term reproducibility (Klausner et al., 2005), despite still undergoing validation testing by ECVAM and ICCVAM.

As of August 2007, ECVAM reports that ten validated alternative methods and animal test deletions have gained regulatory acceptance (ECVAM, 2007):

1) EpiDerm™ skin corrosivity test
2) Rat TER skin corrosivity test
3) Episkin® skin corrosivity test
4) 3T3 NRU phototoxicity test
5) Local Lymph Node Assay (LLNA) for skin sensitization
6) Toxin Binding Inhibition (ToBI) test for batch potency testing of tetanus vaccines for human use
7) ELISA test for batch potency testing of tetanus vaccines for human use
8) ELISA test for batch potency testing of erysipelas vaccines
9) In vitro tests for percutaneous absorption
10) Deletion of the acute oral toxicity test, Lethal Dose (LD50)

EU Directive 86/609/EEC helps ensure that these methods are now used in Europe rather than animal testing methods. Similar legislation in the US would safely expedite implementation of validated alternatives and promote international harmonization.

III.1.4 Funding of Non-Animal Methods

Funding for alternatives research lags far behind that of animal methods. In Germany, where national funding for alternatives is probably highest in the world (Gruber and Hartung, 2004), total investment between 1980 and 2000 was €82 million (~ US $111 million), compared with €456 million (~ US $618 million) invested by the German Research Council alone for the year 2002 (ibid). The UK government recently announced it was awarding £3 million (~ US $6 million) in funds to the National Centre for the Replacement, Refinement and Reduction of Animals in Research for 2006-2008. Comparisons are hard to perform as figures are not readily available, but annual funding for animal procedures performed in the UK must be considerable: annual spending on pharmaceutical R&D in industry is estimated to be £3 billion (~ US $6 billion), with an additional £1 billion (~ US $2 billion) in publicly-funded research.

III.1.5 Benefits of International Harmonization

Harmonization of guidelines pertaining to animal tests that provide safety or efficacy data for regulatory authorities has facilitated the acceptance of single study designs by such bodies in many countries. This has been extremely valuable in ensuring that the minimum number of animals is used on a global level in safety and efficacy testing. It can reduce the need for repeat testing, eliminate redundancy (where more than one test provides the same information), minimize group sizes (e.g., by agreement to use a single sex) and lead to the adoption of shortened protocols, reduced animal numbers and less severe treatments and procedures.

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) had by the 1990s produced an estimated 50 percent average global reduction in animal use for preclinical testing of pharmaceuticals (Roe, 1993). Another major influence has been the OECD Test Guidelines Program, which has developed standardized methods for testing of chemicals

that are accepted in principle by all 30 OECD member countries (Balls, 1994; Balls, 2002; Gad, 2000) through an agreement on the mutual acceptance of data.

Harmonization of testing methods and protocols has clearly been a force for positive change. This mutual acceptance of data has led to vast reductions in animal use, with concomitant benefits for the public in terms of better, more predictive and reliable testing methods. It has impacted skin and eye irritancy testing (Stephens et al., 2002), vaccine safety and efficacy testing (Brown and Levine, 1999), and the deletion of obscure and redundant animal tests from formal requirements.

It is reasonable to conclude that further harmonization, specifically in terms of the validation, approval and implementation of non-animal methods in the US, Europe and Japan, would produce additional benefits regarding human health and animal welfare. For example, ensuring that alternative methods deemed to be scientifically satisfactory by an acknowledged validation agency are also accepted and implemented in the other partner countries by means of legislation, regulation, and international agreements would provide substantial benefits for the public, the pharmaceutical companies, and the animals who would be excluded from testing.

III.2 Problems With Animal Methods

III.2.1 Animal Consumption

United States

A key impetus for using non-animal methods is that this can spare large numbers of animals the pain and distress often involved in animal-based techniques (Balcombe et al., 2004). If superior or even similarly efficacious non-animal methods exist for advancing product safety and scientific knowledge without harming animals, then no further justification should be required for using them.

In the United States, the number of animals affected by this petition is well into the tens of millions and increasing. A recent surge in the numbers of animals used is to a great extent due to a new emphasis on transgenic mice (O'Shea, 2000; Fishbein, 2001). A US laboratory animal veterinarian recently estimated that the number of mice now consumed yearly for US laboratory research exceeds 100 million (Carbone, 2004). This figure represents a dramatic rise over that provided by the Office of Technology Assessment (OTA), which estimated in the mid 1980s that 17 to 22 million vertebrate animals were being used annually (OTA, 1985).

Three US government-initiated chemical testing programs undertaken since 1998 (the High Production Volume Chemical Testing Program [HPV], the Endocrine Disruptor Screening Program [EDSP], and the Voluntary Children's Chemical Evaluation Program [VCCEP]) threaten to greatly increase the numbers of animals being subjected to harmful animal tests. Since its inception, the HPV program has reportedly subjected over 150,000 animals to chemical tests, and not banned or regulated any toxic industrial chemical in the process (Sandusky et al., 2006). The EDSP and VCCEP programs are not yet underway, but they also augur ill for animals—more than a million may be used in lethal test protocols. These programs underscore the urgent need for a mandatory alternatives policy for chemicals and drugs in the US.

European Union

EU data suggest that animal consumption rates may have been declining somewhat across Europe in recent years, based on fairly constant numbers reported from a steadily increasing EU membership. In 1991-1992, the first year of EU reporting, total animal use among 10 member states totaled 11.8 million animals. This figure dropped slightly to 11.6 million in 1996, by which time the EU had grown to 15 member states. In 2002, the latest reporting period, the total reported number of animals used in the 15 member states of the EU was 10.7 million (CEC 2005). Because 2002 marked the first time that harmonized reporting was in effect, it is difficult to be certain whether any trend exists.

It remains to be seen how the increased use of transgenic mice might affect this apparently declining trend in the EU. In Great Britain animal use increased by 1.4% for the latest reported year (2005), culminating in a 10-year high of almost 2.9 million procedures (Home Office, 2006).

III.2.2 Scientific Problems with Animal Use

Extrapolating across species is a tenuous enterprise. Animal test data – which typically involve organ-level or systemic responses – are undermined by species differences in anatomy, organ structure and function, toxin metabolism, chemical absorption, and mechanisms of DNA repair. For example, when rat and mouse carcinogens are compared, the test results for rats are consistent with the test results for mice only two-thirds of the time (Stephens, 2000).

These differences are compounded by additional variables introduced by demographics (e.g., age, sex, strain, genetic and immune status) and by husbandry (e.g., caging, diet, handling). Vastly shorter life spans further complicate the inducement and interpretation of animal responses, particularly for conditions (e.g., cancer and cardiovascular disease) with protracted etiologies and clinical courses in humans.

Such is the sensitivity of caged mice to their surroundings that familiarity with human handlers has been found to significantly affect results (Chesler et al., 2002; Van Driel and Talling, 2005), and painstaking attempts to standardize protocols across laboratories fail to guarantee reproducibility of results (Crabbe, 1999; Würbel, 2002). To the extent that non-animal methods are not subject to these factors, these problems can be avoided.

The extrapolation problem has been widely acknowledged, evidenced by literally hundreds of statements from scientists of many disciplines and affiliations. For example:

- "The methods of assessing toxicity in animals are largely empirical and unvalidated…It is urgently necessary to know whether the tests as in fact conducted have sufficient predictive value to be justifiable, or whether they are a colossal waste of resources to no good purpose."

  Professors Laurence, McLean and Weatherall, writing in the introduction to their book, *Safety Testing of New Drugs - Laboratory Predictions and Clinical Performance*, ed. DR Laurence, AEM McLean & M Weatherall, publ. Academic Press, 1984.

- "Extrapolating from one species to another is fraught with uncertainty…For almost all of the chemicals tested to date, rodent bioassays have not been cost-effective. They give limited and uncertain information on carcinogenicity, generally give no indication of mechanism of action, and require years to complete. [They are] rarely the best approach for deciding whether to classify a chemical as a human carcinogen."

  Dr. Lester Lave, of Carnegie Mellon Univ., and Drs. Ennever, Rosenkrantz and Omenn, writing in *Nature*, Vol 336, p 631, 1988.

- "Surely not even the most zealous toxicologist would deny that epidemiology, and epidemiology alone, has indicted and incriminated the cigarette as a potent carcinogenic agent, or would claim that experimental animal toxicology could ever have done the job with the same definition."

  Dr. Michael Utidjian, writing in *Perspectives in Basic and Applied Toxicology*, p 309-329, ed. Bryan Ballantyne, publ. Butterworth, 1988.

- "The standard carcinogen tests that use rodents are an obsolescent relic of the ignorance of past decades."

  Philip Abelson, Editor of *Science*. *Science* (1990), Sep 21, p 1357.

- "We always have a battle on the issue of what to do with the animal data."

  Dr. Edward Stein, Health Scientist, US Occupational Safety and Health Administration (Brinkley, 1993).

- "So much evidence has accumulated that chemicals frequently have wholly different effects in animals and humans that officials throughout government and industry often do not act on the studies' findings."

  Brinkley, Joel, *New York Times*, "Many say lab-animal tests fail to measure human risk," March 23, 1993, p A1.

- "We have relied too heavily on animal testing, and we believed in it too strongly. Now, I think we are commencing to realize that what goes on in an animal may not necessarily be applicable to humans."

  Marvin Pollard, former president of the American Cancer Society. *Expressions* 2 (1994) New England Anti-Vivisection Society, p4. (Available from New England Anti-Vivisection Society: http://neavs.org)

- "In the face of these shortcomings, many experts believe the scientific value of the 2-year bioassay is highly limited—barely worth the investments in personnel, animals, money, and time."

  Charles W. Schmidt (Schmidt CW, 2002).

- "Most of the animal tests we accept have never been validated. They evolved over the past 20 years, and the FDA is comfortable with them."

  Anita O'Connor, FDA. Personal communication, cited in "Monkeying Around with Human Health;" Animal Aid, June 2004: http://www.animalaid.org.uk/images/pdf/primates.pdf.

- "It is difficult to translate animal experiments into humans. Drugs are notorious for having other effects."

  "We have to temper our enthusiasm with a high dose of reality, making sure we understand the pitfalls in animal studies."

  David Park, associate professor of neuroscience, University of Ottawa. (E-healthsource, May 17th 2005: http://news.e-healthsource.com/index.php?p=news1&id=525709).

- "Currently, nine out of ten experimental drugs fail in clinical studies because we cannot accurately predict how they will behave in people based on laboratory and animal studies"

  Mike Leavitt, Secretary of Health and Human Services, U.S. Department of Health and Human Services (Food and Drug Administration press release, *FDA Issues Advice to Make Earliest Stages of Clinical Drug Development More Efficient*, January 12, 2006).

- "Consider just one stark statistic: Today, nine out of 10 compounds developed in the lab fail in human studies. They fail, in large part because they behave differently in people than they did in animal or laboratory tests."

  Andrew C. von Eschenbach, M.D. then Acting Commissioner of the FDA (prepared statement for FDA Teleconference: *Steps to Advance the Earliest Phases of Clinical Research in the Development of Innovative Medical Treatments*, January 12, 2006).

These problems manifest repeatedly when attempting to apply animal data to human clinical conditions. There are numerous examples of animal studies providing misleading information, with false favorable results contributing to human morbidity and mortality, and false unfavorable results delaying or preventing the use of beneficial treatments.

A few brief examples below illustrate this problem. Much of the list includes examples of drugs that elicit different responses in animals and humans, although the situation is much graver than one might infer from these alone. An astounding 92 percent of drugs that enter clinical trials, having obtained Investigative New Drug approvals largely on the basis of data from animal testing, fail to obtain FDA New Drug Application approval for marketing (Harding, 2004). Failure rates for some categories of drugs are even higher, such as the 95 percent failure rate for cancer drugs (Kola, 2004). Such a high attrition rate does not demonstrate preclinical testing that is rigorous, appropriate and reliable.

Of the eight percent of drugs entering human clinical trials that gain FDA approval, half are withdrawn or relabeled post-marketing due to severe or lethal adverse effects not detected in animal tests. These approved drugs are directly responsible for a level of adverse drug reactions that constitutes (depending on how the figures are calculated) the fourth or fifth leading cause of death in the US (Lazarou, 1998), with a similar impact in the UK (Pirmohamed et al., 2004).

These statistics and examples are not surprising considering comprehensive studies of comparative drug toxicology, which have revealed levels of discordance between results from animals and humans of between 67 and 96 percent (Lumley and Walker, 1990; Spriet-Pourra and Auriche, 1994). These studies show that animal-based toxicology is not predictive of human response, providing correct predictions less often than a coin toss.

Many experts from academia and industry have gone on record supporting this view, and advocating the use of alternative and more predictive scientific methods in drug development (Bailey, 2005b):

1. In 2002 the US Women's Health Initiative (WHI) abruptly ended its flagship clinical trial of a hormone replacement therapy (HRT) when it was discovered that the 8,000-plus women taking a popular estrogen and progestin combination pill were more likely to have heart attacks, strokes, vascular thrombosis, and breast cancer than were the women in the placebo group. Decades of prior research on mice, rabbits, pigs, and monkeys had shown only beneficial effects. HRT is estimated to have caused over 20,000 *additional* cases of breast cancer in the UK in the last decade (Beral et al., 2003), and with 38 percent of American postmenopausal women on HRT, the impact in the US will have been much greater.

2. The initial development of protease inhibitors – drugs that significantly decrease HIV death rates – was compromised by a reliance on disappointing animal tests, which precipitated a four-year delay in the start of clinical trials. Tens of thousands of HIV-related deaths might have been avoided had this delay not occurred (Tatchell, 2004).

3. The arthritis drug Vioxx appeared to be safe and even to provide cardiovascular protection in animal studies, but was withdrawn from the global market in September 2004 after causing as many as 140,000 heart attacks and strokes and over 60,000 deaths in the US alone (Graham et al., 2005). Globally, the figures are estimated to be 320,000 heart attacks and strokes, and 140,000 deaths (Graham et al., 2005; Topol, 2004), making Vioxx easily the most lethal prescription drug ever approved.

4. A recent review and analysis of animal-based teratology studies documented mean positive and negative predictive values for human clinical outcomes only slightly above 50 percent, and substantial discordance among the species used (Bailey et al., 2005*a*). This review looked only at correlation of *definitions* (i.e., presence or absence of teratogenic effects) – if *specific* teratogenic effects had been examined, the correlation would have been even lower.

5. Smoking's link to lung cancer first became scientifically evident from two landmark epidemiological studies published in 1950 (Wynder and Graham, 1950; Doll and Hill, 1950). Since then, tens of thousands of subjects in clinical studies have reinforced that link. During the same period and continuing even today, legions of animal studies of tobacco and tobacco smoke have been performed. Their general failure to reflect human sensitivity from exposure to tobacco smoke renders them poor models for predicting human risk (Little, 1961; Ames et al., 1987; Coggins, 2002). False negative animal smoking studies delayed public health warnings against cigarette smoking for years, contributing to untold numbers of preventable human deaths (Anderegg et al., 2006).

6. Since their commercial introduction in the early 1980s, many non-steroidal anti-inflammatory drugs (NSAIDs) have been clinical failures. Found safe in year-long studies in rhesus monkeys, benoxaprofen produced thousands of serious adverse events and dozens of deaths within three months of approval and marketing (Dahl and Ward, 1982). Fenclofenac showed no toxicity in ten animal species, yet produced severe liver toxicity in humans and was withdrawn (Gad, 1990). Similar fates befell

NSAIDs phenylbutazone (Venning, 1983), suprofen (Heywood, 1990), zomepirac (Ross-Degnan et al., 1993), and bromfenac (Peters, 2005) after animal tests failed to predict harmful effects in humans.

7. Many other categories of drugs have been safe and effective in animal studies, yet were harmful enough to be withdrawn or relabeled for serious adverse effects in humans. These include antibiotics such as chloramphenicol (Wallenstein and Snyder, 1952; "Danger of chloramphenicol," 1952), clindamycin (Gray et al., 1972; Venning, 1983), and temafloxacin (Krasula and Pernet, 1991; Blum et al., 1994); anti-virals such as idoxuridine (Marks, 1975; Harrison et al., 1996); antidepressants such as nomifensine (Fielding and Szewczak, 1984)); cardiovascular medications such as ticrynafen (Acosta et al., 1982; Manier et al., 1982), mibefradil (Mulder et al., 1998; Bernardeau et al., 2000), amrinone (Eason et al., 1990), and cerivastatin (von Keutz and Schluter, 1998), among many others.

8. There are many useful, safe human drugs that would not currently survive animal testing because of severe or lethal toxicities in some species. Among the more notable examples are penicillin (Schneierson and Perlman, 1956; Millen, 1962; Koppanyi and Avery, 1966), acetaminophen (Savides et al., 1984; Hjelle and Grauer, 1986; Villar et al., 1998), and aspirin (McColl, 1967; Wilson and Gavan, 1967; Khera, 1976; Wilson et al., 1977; Robertson et al., 1979; Sanders and Stephens, 1991).

9. More than 50 preventive vaccines and more than 30 therapeutic vaccines for HIV/AIDS have been successful in non-human primate studies, yet every one has failed in human trials completed between 1987 and 2006 (www.clinicaltrials.gov[15] and http://www.niaid.nih.gov/factsheets/clinrsch.htm). This abject failure of translation to human results explains why AIDS researcher Margaret Johnston stated: "HIV/AIDS [animal] models have not yielded a clear correlate of immunity nor given consistent results on the potential efficacy of various vaccine approaches" (Johnston, 2000).

10. In the early 1980s, the observation that HIV does not affect the chimpanzee led to the assumption that the virus was harmless to humans too. Health authorities were subsequently advised to allow transfusion with contaminated blood samples, the very cause of the French blood scandal that claimed thousands of innocent victims. This was referred to in the 1996 inquiry into mad cow disease in the UK, when Pierre Tambourin (then head of the life science department at the National Center for Scientific Research, CNRS, in France) stated, "What are the chances of developing a prion disease following ingestion of contaminated meat? Nobody knows, but we must not repeat the error we did in 1983-1985 with AIDS, when we referred to animal

---

[15] ***Preventive*** HIV vaccine trials: current or pending
http://www.clinicaltrials.gov/ct/search;jsessionid=D9B6FD8A949F7D545382387828812432?term=%22hiv+preventive+vaccines%22&submit=Search
Preventive HIV vaccine trials: completed, terminated or no longer recruiting
http://www.clinicaltrials.gov/ct/search?term=%22HIV+Preventive+Vaccine%22+%5BCONDITION%5D+AND+%28+%22No+longer+Recruiting%22+OR+%22Completed%22+OR+%22Terminated%22+%29+%5BOVERALL-STATUS%5D&submit=Search
Therapeutic HIV vaccine trials: current or pending
http://www.clinicaltrials.gov/ct/search?term=%22hiv+therapeutic+vaccine%22
Therapeutic HIV vaccine trials: completed, terminated or no longer recruiting
http://www.clinicaltrials.gov/ct/search?term=%22HIV+Therapeutic+Vaccine%22+%5BCONDITION%5D+AND+%28+%22No+longer+Recruiting%22+OR+%22Completed%22+OR+%22Terminated%22+%29+%5BOVERALL-STATUS%5D&submit=Search (All accessed January 10 2007).

models to dramatically underestimate the risk to which humans are exposed" (National Assembly, 1996).

11. Over 4,000 studies have been reported demonstrating the efficacy of more than 700 drugs in animal models of stroke (Macleod et al., 2004). About 150 of these drugs have been tested in human clinical trials, and all have failed to show benefit (Macleod, 2005). Only recombinant human tissue plasminogen activator (rt-PA) administered within three hours of stroke onset has shown symptomatic benefits, but it has been associated with 10 times as many intracerebral hemorrhages and it produces no survival advantage (National Institute of Neurological Disorders and Stroke, 1995).

12. The entire field of cancer immunology animal research has failed to produce even one successful therapeutic cancer vaccine, and the paradigm has been criticized by the Chief of the Surgery Branch of NCI. In a review of clinical trials showing no therapeutic benefit from cancer vaccines, Dr. Steven A. Rosenberg wrote: "In the light of these very large numbers of patients treated with vaccines and the exceedingly low objective response rates reported for the cancer types included in Table 5, a reevaluation of future directions for cancer immunotherapy trials would be valuable." He also highlighted the harmful effects of the falsely optimistic reports from investigators and media that often accompany these trials: "The ineffectiveness of cancer vaccine approaches is not commonly appreciated, however, because of the 'spin' often accompanying reports of cancer vaccines." (Rosenberg et al., 2004).

The inherent unreliability of animal-based studies can also be seen by how animal and human studies proceed independently of one another. To ensure human safety, animal studies are meant to be conducted before human clinical trials. However, a recent review of six animal trials found that in two cases clinical trials were conducted concurrently with the animal studies, in three cases clinical trials were conducted despite evidence of harm from prior animal studies, and in the remaining case the outcome of the animal study contradicted the findings of previous investigators, who appeared to have cited only studies that supported their views (Pound et al., 2004).

A similar review completed in 2006 (Perel et al., 2006) evaluated the extent to which animal experiments correlate with the human clinical situation, and found: (1) animal tests fail to reliably predict effects in humans; (2) many animal experiments are of poor quality, and; (3) the results of animal tests are not being adequately communicated to those conducting later clinical trials.

Six interventions (drug treatments for brain injury and blood loss, two stroke treatments, and preventive treatments for lung damage in premature babies and osteoporosis in women) were investigated in the Perel review, and associated animal studies were assessed statistically regarding their agreement with human studies. In all six cases, involving 176 animal-based research studies, the researchers heavily criticized the quality of the animal studies. In four of the six interventions the animal studies failed to correctly predict the human outcome. In two of these cases they actually predicted a beneficial effect when the treatment was ineffective and harmful to humans. The researchers also often reported finding animal studies that had been conducted at the same time or even after the human studies had shown the treatment to be effective, and that the results of

animal studies were not effectively being incorporated into human research – the key justification for conducting them in the first place.

A recent analysis of potential carcinogens also found poor correlation of cancer risk between the US EPA and the International Agency for Research on Cancer (IARC). For 128 chemicals with human or animal data assessed by both agencies, human carcinogenicity classifications were similar only for those 17 having significant human data. For 111 chemicals whose carcinogenicity ratings were primarily based on animal data, the EPA was far likelier than the IARC to assign human carcinogen classification (Knight et al., 2005). In fact, in 60 percent of cases animal data were found to be insufficient to enable a classification of human risk.

The use of genetically modified (GM) animals has resulted in a drastic increase in the numbers of animals used in research over the past decade. However, research involving these animals has been beset by the same problems as research involving their non-GM counterparts. Transgenic animal models used for research on cystic fibrosis, Alzheimer's disease, Parkinson's disease, cancer, and diabetes, among other diseases, have revealed a widespread failure to duplicate human symptoms characteristic of those conditions. Even with identical genetic mutations these transgenic animal models show poor correlation with the actual human diseases, and thus they are unlikely to enable scientists to use them to elucidate the molecular processes underlying those diseases and to develop effective treatments (Bailey, 2005*c*).

III.2.3 Costs

In addition to scientific and humane incentives, there are also substantial economic advantages to the adoption of replacement methods for animal tests. Animal-based methods are routinely very costly. The 24-Month Rat Inhalation Toxicity assay prescribed by *The Handbook of Toxicology*, Second Edition, is listed as typically costing $1.4 million to complete (Derelanko and Hollinger, 2002). The current gold standard for testing a compound to determine if it is carcinogenic is the rodent bioassay, which takes around five years from planning to evaluation and review, at a cost of up to more than $4 million per substance (NTP, 2006). And if animal-based testing methods are used, the EU's 2001 REACH (Registration, Evaluation and Authorization of CHemicals) program – aimed at harmonizing testing requirements for about 30,000 chemicals marketed before September 1981 – will consume an estimated 12.8 million animals at an estimated cost of €8.68 billion (~ US $11.7 billion) (Hartung et al., 2003). That represents about $390,000 (US) for each chemical tested.

It is widely known that most replacement methods cost less and take less time to complete than animal-based methods. DakDak, for example, is a test used to measure the effectiveness of sunscreens in preventing skin damage. Charles River Laboratories (CRL), which purchased DakDak in 2002, reports that this test does in days what it takes animal studies months to do, and CRL estimates that it can test five or six products for less than half the cost to study one product in animals (Aoki, 2002). Another test being used by CRL (though not a full replacement) is the Limulus Amebocyte Lysate (LAL) test for pyrogenicity, which monitors the manufacturing process for toxins. Approved by regulators, LAL is cheaper, faster, and easier to run than animal methods (ibid), but uses and harms *Limulus* during the assay. Encouragingly however, five new *bona fide* replacement pyrogenicity tests that can be used in place of the LAL test were validated by

ECVAM in 2006. Replacement methods also save on various hidden costs associated with animal methods, including animal procurement, maintenance and husbandry, and hazardous waste disposal.

A less publicized cost of laboratory research using animals is the waste generated by discarding the bodies of dead, unused animals. An estimated 75 percent of the 100 million transgenic mice bred for use in laboratory research are killed because they are unusable or not needed in research protocols (Carbone, 2004). A primary reason for this is that getting the right genetic profile is a hit-and-miss process.

Aside from the actual costs of the methods themselves, non-animal methods can yield other cost savings. For example, in vitro screening methods allow companies to narrow a list of promising test materials in a cost- and time-efficient manner. Companies typically need to screen a very large number of drug candidates, such that conducting animal tests for each is beyond their budget. Using an in vitro screen, however, they can test the whole set and narrow the field to the most promising candidates before progressing to the very expensive human clinical trials (Rodger Curren, Institute for *In Vitro* Sciences, personal communication, July 2005).

Finally, a discussion of costs associated with animal methods would be incomplete without mention of the potential for costly legal claims against companies that rely on animal data. This sort of claim was hypothetical until July 2005, when the non-profit organization Physicians Committee for Responsible Medicine charged the drug maker Merck and Co., Inc. with improperly relying on animal tests to show that their arthritis drug Vioxx was safe for humans. Plaintiff Nancy Tufford, a Minnesota resident who claims that Vioxx caused her to develop congestive heart failure, is seeking $1 million in damages from the company (Silverman, 2005). While the outcome of this lawsuit may not be known for years, it sets a precedent that is almost certain to be followed, given current reliance on animal preclinical studies and the frequency with which new pharmaceuticals damage human patients (**Subsection B.*III.2.2***, above).

US government agencies expose themselves to litigation stemming from an over-reliance on and/or improper use of animal testing. The EPA, for example, has been the target of several recent and current lawsuits. These include two suits challenging the EPA's animal testing schemes for its High Production Volume (HPV) Chemicals testing program, a current suit concerning the Developmental Neurotoxicity Test (DNT), and another concerning the EPA's Endocrine Disruptor Screening Program (EDSP). Each of these suits faults the agency for failing to carry out various congressional mandates.

For example, the methods described in EPA's DNT Guidelines have never been subject to formal or adequate scientific validation to verify that the results of this animal test are reliable and relevant predictors of real-world effects in human beings. Furthermore, the EPA has used DNT test results to justify allowing children (those in the womb, nursing infants, and growing children) to be exposed to pesticide levels many times higher than the statutory tenfold "children's health safety factor" established by Congress under the Food Quality Protection Act of 1996. These unreliable and improperly used animal tests represent a costly and time-consuming burden that could be avoided by proactive development and use of more reliable, predictive and humane non-animal methods.

IV. Comparison of US and European Law

IV.1 US Law

Excerpts of US laws relevant to this petition are set forth in **Appendix B**.

IV.2 European Law

Excerpts of European laws relevant to this petition are set forth in **Appendix B**.

V. Support for this Petition

V.1 The Public

Throughout recent history, it has repeatedly been shown that the US public has serious reservations about animal testing and experimentation, particularly when it involves animal pain and distress and/or the testing of non-essential products. In a survey released in 1990, 60 percent of a sample of 1,000 American adults opposed the use of animals in cosmetics testing, while 43 percent and 20 percent opposed animal testing of over-the-counter medicines and prescription drugs, respectively (Ward, 1990). A survey of 757 Americans conducted in September 2001 by an independent polling firm found that 75 percent of people disapprove, and most strongly disapprove, of experiments that subject animals to severe pain and distress (HSUS, 2001). Sixty percent of respondents opposed research and testing that cause even moderate pain and distress, and 33 percent were opposed even when little or no pain or distress was involved. An independent survey conducted by the Humane Research Council, a national consumer and market research company, showed that 71 percent of the American public believes that chimpanzees used for more than 10 years in research should be retired, amounting to approximately 76 percent (987 of the estimated 1,300) of chimpanzees in US laboratories (Project R&R, 2006).

These polls are congruent with other opinion polls on animal experimentation, which show that support declines dramatically when the potential benefits of the research are deemed low, the type of animal involved is considered highly sentient, and the level of invasiveness is high (Plous, undated). This suggests widespread public support for the aim of this petition: that companies and investigators be *required* to use replacement methods when they are available and proven scientifically satisfactory.

Given an educated choice, the public expresses a strong preference for non-animal-tested products. In a national poll conducted by CARAVAN® Opinion Research Corporation in 1996, respondents overwhelmingly stated that they would be more likely to buy a product if they had an indication that the product was not tested on animals (CCIC Fact Sheet, 1996).

V.2 Scientists

Broad scientific support for non-animal methods is evident in the shift to non-animal testing evidenced above, in the inexorable growth and diversification of promising methods and of those that have achieved validation, and in the statements of many scientists, examples of which are given in **Subsection B.*III.2.2***. The success of the World

Congress on Alternatives and Animal Use in the Life Sciences is another indicator of the scientific robustness of the field. This meeting was inaugurated in Baltimore in 1993, and since has been held in Utrecht, Bologna, New Orleans, Berlin, and Tokyo. The Sixth World Congress in Tokyo from August 21-25, 2007 attracted more than 950 participants from around the world to discuss a broad range of topics, including ways to advance the legislative agenda.

Appendix C presents a list of scientists and other experts who support this petition.

In addition, an independent poll of general practitioners in the United Kingdom conducted in 2004 (TNS Healthcare, 2004), showed that 82 percent of physicians "at the front line" prescribing drugs are concerned that animal tests are misleading when applied to humans.

C. ENVIRONMENTAL IMPACT

The proposed regulatory and policy changes will have a favorable environmental impact because they will result in many fewer animals being used for drug and device testing in the US, which in turn will greatly diminish air, soil, and water contamination caused by the use and disposal of many millions of such animals annually. Environmental and human health hazards related to animal testing occur by means of atmospheric release of incinerator stack gases and particulates, deposition of numerous soil contaminants, and entry of waste and toxins into groundwater, lakes and rivers, and drinking water.

Air contamination is produced by the emission of gases and particulates resulting from incineration of animal carcasses that typically contain experimental chemicals, drugs, and other toxins, often at concentrations many times greater than would be tolerated by humans. The resulting release of toxic substances is related in part to processes common to all industrial incinerators, and in part to toxins specifically produced by incineration of animal carcasses. There is very little information regarding the potential hazards of airborne substances related specifically to the drugs, chemicals, and other toxins in the animal carcasses, particularly since in most cases there are no reliable detection methods.

Incinerator gases such as sulfur dioxide, carbon monoxide, and nitrogen oxide can cause or exacerbate respiratory and cardiovascular diseases such as asthma, bronchitis, heart attack and stroke (D'Amato et al., 2005; Bernstein et al., 2004; Maynard, 2004). These emissions also decrease resistance to infections and contribute importantly to smog, acid rain, and ozone formation (Rowat, 1999). Exposure to airborne incinerator particulates is associated with increased risks for asthma, hypertension, stroke, and cardiac diseases (D'Amato, et al., 2005; Brook, 2005), as well as with increased mortality (Krewski, et al., 2003; Brook, 2005).

Stack gases from animal carcass incinerators contain higher concentrations of toxic heavy metals than standard medical waste incinerators, including iron, copper, zinc, lead, nickel, and manganese (Chen et al., 2004). Polycyclic aromatic hydrocarbons (PAH) are also emitted in animal incinerator stack gases, and the concentrations of the most carcinogenic PAH compounds are reported to be 4.6-7.6 times greater than in standard medical waste incinerators (Chen et al., 2003).

Soil contamination related to animal testing occurs from both incinerator residues and water runoff from testing facilities. In several studies, increased levels of heavy metals, dioxins, and polychlorinated dibenzofurans were present in the soil near incinerators (Oh, et al., 2006; Segura-Munoz, et al., 2004; Jimenez, et al., 1996). The specific dioxin 2,3,7,8-TCDD, a byproduct of incomplete combustion, is one of the most toxic chemicals known, and according to IARC a definite human carcinogen (Mandal, 2005). Animal incinerator soil contaminants in bottom ash and fly ash also include calcium, phosphorus, and potassium, which can have toxic effects (Thompson, et al., 1995).

Ground water contamination is caused secondarily by soil contamination, and also by the runoff of drug- and toxin-containing animal waste and other debris related to drug and chemical testing. The growing problem of drugs in public water supplies is exacerbated by the contributions from animal waste containing drugs and chemicals that may have unknown toxicities due to their experimental nature. A 2002 landmark study by the US Geological Survey found that 80 percent of sampled rivers and streams contained one or more pharmaceuticals (USGS, 2002), and it is expected that in 2008 the EPA will begin monitoring the presence of pharmaceuticals in water supplies (Cone, 2006).

Public drinking water supplies are contaminated through animal testing because public water treatment facilities are almost universally incapable of filtering out the drugs, hormones, and some chemical solvents in the waste water from animal testing facilities. According to the EPA and many environmental studies in the U.S. and Europe, treated waste water carries these potential toxins into the surface water, groundwater tables, streams, rivers, lakes and aquifers ─ and thus into the public drinking water supply.

There are related serious biological consequences for aquatic animals, and potentially serious health effects for humans, from the presence of antibiotics, endocrine disruptors, cytotoxic cancer drugs, and other drugs in lakes, rivers, streams, and drinking water (CBC News, 2006; Daughton and Ternes, 1999). A 2006 Italian study evaluated the effects of a cocktail of drugs (designed to mimic river and treated waste water content) on human kidney cells, and found that cellular proliferation was reduced 10-30 percent compared to control cells (Pomati, et al., 2006).

Granting this petition will gradually decrease the numbers of animals used for drug and device testing, and thereby mitigate the known and suspected serious environmental and health consequences described in this section.

D. ECONOMIC IMPACT

The economic advantages from the adoption of replacement methods for animal tests are discussed in **Subsection B.*III.2.3*** above, which summarizes the costs of animal-based strategies and provides examples of less expensive and faster non-animal approaches.

In addition, **Subsection B.*III.2.3*** highlights other substantial cost savings from advanced in vitro screening methods, such as rapidly and effectively narrowing panels of candidate drugs to select those with most promise. Increased litigation directed at pharmaceutical and chemical companies, as well as government agencies, has economic costs for shareholders, consumers and taxpayers – such as lawsuits against Merck and Co., Inc. regarding Vioxx, and against the EPA for some of their testing programs. Finally, the

adoption of replacement methods would eliminate the costs of otherwise disposing of millions of animals that would have been used or wasted in the processes.

E. CONCLUSION

Promulgation and enforcement of policies mandating the use of scientifically satisfactory non-animal methods for drug and device testing provide a number of benefits, including:

- Developing test methods that are more applicable and beneficial to human health
- Sparing animals significant amounts of pain and suffering, as well as death
- Lowering costs for companies and consumers
- Boosting incentives for development, validation and adoption of alternative methods in research and education as well as testing
- Helping create markets for new methods, which must be used once available
- Providing a new, clear-cut criterion for evaluating compliance with the law
- Strengthening ICCVAM's mandate, which currently cannot require federal agencies to adopt validated methods
- Bolstering USDA APHIS Animal Care Manual Policy 12 (search for alternatives)
- Fostering and fortifying state and congressional activities in support of non-animal alternatives development, validation and adoption
- Avoiding lawsuits that impair drug development and increase development costs

The time is appropriate for these regulatory changes regarding non-animal test methods to be incorporated into company and FDA practices. As long as the FDA merely *suggests* that alternatives be considered, and *permits* submission of non-animal test data, there is little or no incentive to change the status quo. There are many sound scientific, economic, and humane reasons for replacing animal methods with scientifically satisfactory alternatives. There is also a time-tested successful legislative precedent in the EU (Directive 86/609/EEC). Requiring the use of scientifically satisfactory non-animal test methods is a vital step if the US is to keep pace with Europe in advancing the broad goal of replacing methods that harm or kill animals with more reliable, efficient, and humane methods of testing, research and education.

F. CERTIFICATION

The undersigned certifies, that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which the petition relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.

(Signature) _____

(Name of petitioner) _____

(Mailing address) _____

(Telephone number) _____

LITERATURE CITED

Acosta D, Mitchell DB, Santone KS, Bock A, Lewis W (1982). Lack of cytotoxicity of ticrynafen in primary cultures of rat liver cells. *Toxicology Letters* 10:385-8.

Ames BN, Magaw R, Gold LS (1987). Ranking possible carcinogenic hazards. *Science* 236:271-80.

Anderegg C, Archibald K, Bailey J, Cohen MJ, Kaufman SR, Pippin JJ (2006). A critical look at animal experimentation. Medical Research Modernization Committee, Cleveland, Ohio. [Accessed May 29 2007] Available at: http://www.mrmcmed.org/Critical_Look.pdf

Anonymous (1999). Proceedings of the Production of Monoclonal Antibodies Workshop, August 29, Bologna, Italy. [Accessed May 29 2007] Available at: http://altweb.jhsph.edu/topics/mabs/ardf/intro.htm

Aoki N (2002). Evolving away from animal tests. *Boston Globe*, 27 February.

APHIS Animal Care Program Inspection and Enforcement Activities, Audit Report No. 33002-3-SF (September 2005). [Accessed May 29 2007] Available at: http://www.usda.gov/oig/webdocs/33002-03-SF.pdf

Australian Government, Department of Agriculture Fisheries, and Forestry. National Consultative Committee on Animal Welfare (NCCAW) Position Statement (October 1993). [Accessed May 29 2007] Available at: http://www.daffa.gov.au/animal-plant-health/welfare/nccaw/guidelines/research/draize

Bailey J, Knight A, Balcombe J (2005*a*). The future of teratology research is *in vitro. Biogenic Amines* 19:97–145.

Bailey J (2005*b*). Non-Human Primates in Medical Research and Drug Development: A Critical Review. *Biogenic Amines – Stress and Neuroprotection* 19:235-55.

Bailey J (2005*c*). Man or Mouse: Genetically Modified Animals in Medical Research – A Critical Review. Animal Aid, UK. [Accessed May 29 2007] Available at: http://www.animalaid.org.uk/images/pdf/manmouse.pdf

Balcombe JP (2000). *The Use of Animals in Higher Education: Problems, Alternatives, and Recommendations*. Washington, D.C.: Humane Society Press.

Balcombe JP (2001). Dissection: The Scientific Case for Alternatives. *Journal of Applied Animal Welfare Science* 4:118-26.

Balcombe JP, Barnard N, Sandusky C (2004). Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science* 43:42-51.

Balls M (1994). Replacement of animal procedures: alternatives in research, education and testing. *Lab Animal* 28:193–211.

Balls M, Combes R (2005). The Need for a Formal Invalidation Process for Animal and Non-animal Tests. *ATLA* 33:299-308.

Balls M, van Zeller AM, Halder ME. (eds). (2000). Progress in the Reduction, Refinement and Replacement of Animal Experimentation: Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, (29 Aug to 2 Sept 1999, Bologna, Italy), Vol. 31A and 31B. Amsterdam: Elsevier.

Balls M (2002). Future improvements: replacement *in vitro* methods *ILAR J* 43 (Supp l):569–73.

Balls M, Firmani D, Rowan A (eds). (2004). *The Three Rs at the Beginning of the 21st Century: Proceedings of the Fourth World Congress on Alternatives and Animal Use in the Life Sciences*, (11-15 August 2002, New Orleans, USA). *ATLA* 32, Supplement 1 & 2.

Balls M (2004). Are animal tests inherently valid? *ATLA* 32 (Supp 1):755-8.

Beral V; Million Women Study Collaborators (2003). Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 362:419-27.

Benardeau A, Weissenburger J, Hondeghem L, Ertel EA (2000). Effects of the T-type Ca(2+) channel blocker mibefradil on repolarization of guinea pig, rabbit, dog, monkey, and human cardiac tissue. *Journal of Pharmacology and Experimental Therapeutics* 292:561-75.

Bernstein JA, Alexis N, Barnes C, Bernstein IL, Bernstein JA, Nel A, Peden D, Diaz-Sanchez D, Tarlo SM, Williams PB (2004). Health effects of air pollution. *Journal of Allergy and Clinical Immunology* 114:1116-23.

Blower PE, Yang C, Fligner MA, et al. (2002). Pharmacogenomic analysis: correlating molecular substructure classes with microarray gene expression data. *Pharmacogenomics Journal* 2:259-71.

Blum MD, Graham DJ, McCloskey CA (1994). Temafloxacin syndrome: review of 95 cases. *Clinical Infectious Diseases* 18:946-50.

Brindle JT, Antti H, Holmes E, et al. (2002). Rapid and non-invasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabolomics. *Nature Medicine* 12:1439-45.

Brinkley J (1993). 'Many Say Lab-Animal Tests Fail to Measure Human Risk.' *New York Times*, May 23, 1993 (A-1).

Brook RD (2005). You are what you breathe: evidence linking air pollution and blood pressure. *Current Hypertension Reports* 7:427-34.

Brown AP and Levine BS (1999). Relationship Between Dosing Vehicles, Dose Volume, and Stress. Report prepared for the US National Toxicology Program Unpublished report. [Accessed May 29 2007] Available at:
http://www.hsus.org/web-files/PDF/ARI/ARIS_Levine_Toxpaper.pdf

Bruner LH, Carr GJ, Chamberlain M, Curren RD (1996). Validation of alternative methods for toxicity testing. *Toxicology In Vitro* 10:479-501.

CAAT (Center for Alternatives to Animal Testing) (undated). [Accessed May 29 2007] Available at: http://caat.jhsph.edu/publications/animal_alternatives/appendices/d.htm

CCIC (Coalition for Consumer Information on Cosmetics) (2005). Shopping Guide. [Accessed May 29 2007]
Available at:http://www.leapingbunny.org/images/cciclist_05_04_2007.pdf

CEC (Commission of the European Communities) (2005). Fourth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union. Brussels.

Carbone L (2004). *What Animals Want: Expertise and Advocacy in Laboratory Animal Welfare Policy.* Oxford: Oxford University Press.

CBC News (2006). Pill-popping society fouling our water. Telecast March 27, 2006. Available online at http://www.cbc.ca/health/story/2006/03/23/drugs-060323.html

Chang JC, Wooten EC, Tsimelzon A, et al. (2003). Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 362:362–9.

Chen S-J, Hsieh L-T, Chiu S-C (2003). Emission of polycyclic aromatic hydrocarbons from animal carcass incinerators. *Science of the Total Environment* 313:61-76.

Chen S-J, Hung M-C, Huang K-L, Hwang W-I (2004). Emission of heavy metals from animal carcass incinerators in Taiwan. *Chemosphere* 55:1197-1205.

Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, Mogil JS (2002). Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. *Neuroscience and Biobehavioral Reviews* 26:907-23.

Chung CH, Bernard PS, Perou CM (2002). Molecular portraits and the family tree of cancer *Nature Genetics* (Supp 32):533-40.

Clemedson C, McFarlane-Abdulla E, Andersson M, et al. (1996). MEIC evaluation of acute systemic toxicity. *ATLA* 24:273-311.

Clemedson C, Blaauboer B, Castell J, et al. (2005). Acutetox - optimisation and pre-validation of an *in-vitro* test strategy for predicting human acute toxicity. *ALTEX* 23 (Supp l):254-8.

Coggins CRE (2002). A minireview of chronic animal inhalation studies with mainstream cigarette smoke. *Inhalation Toxicology* 14:991-1002.

Combes R., Schechtman L., Stokes W.S, Blakey, D. The International Symposium on Regulatory Testing and Animal Welfare: Recommendations on Best Scientific Practices for Subchronic/Chronic Toxicity and Carcinogenicity Testing. ILAR Journal V43 Supplement 2002. [Accessed May 29 2007] Available at: http://dels.nas.edu/ilar_n/ilarjournal/43_supp/v43supCombes.pdf

Cone M (2006). Traces of prescription drugs found in Southland aquifers. *Los Angeles Times*; January 30, 2006 (B-1).

Crabbe JC, Wahlsten D, Dudek BC (1999). Genetics of mouse behavior: Interactions with laboratory environment. *Science* 284:1670-72.

CREAM trial (Consortium for Resourcing and Evaluating AMS Microdosing). [Accessed May 29 2007] Available at: http://www.xceleron.com/metadot/index.pl?id=2224&isa=Newsitem&op=show

Curren RD and Harbell JW (2002). Ocular safety: A silent (*In Vitro*) success story. *ATLA* 30:69-74.

Dahl SL and Ward JR (1982). Pharmacology, clinical efficacy, and adverse effects of the nonsteroidal anti-inflammatory agent benoxaprofen. *Pharmacotherapy* 2:354-66.

D'Amato G, Liccardi G, D'Amato M, Holgate S (2005). Environmental risk factors and allergic bronchial asthma. *Clinical & Experimental Allergy* 35:1113-24.

Danger of chloramphenicol (no authors listed) (1952). *British Medical Journal* 2:136-8.

Daughton CG, Ternes TA (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives* 107(Supp 6):907-38.

De Boo J, Knight A (2004). Comparative studies of student performance: Humane teaching alternatives demonstrate superior educational efficacy to harmful animal use. [Accessed May 29 2007] Available at: http://www.eurca.org/downloads/animaled/comp.doc

Derelanko MJ and Hollinger MA (eds.) (2002). *Handbook of Toxicology, Second Edition*; Washington, DC: CRC Press.

DTP (Developmental Therapeutics Program) *a*. "DTP Human tumor cell line screen," part of the *In Vitro* Cell Line Screening Project. [Accessed May 29 2007] Available at: http://dtp.nci.nih.gov/branches/btb/ivclsp.html

DTP (Developmental Therapeutics Program) *b*. "NCI AIDS Antiviral Screen," part of the DTP's Screening Technologies Branch. [Accessed June 26 2007] Available at: http://dtp.nci.nih.gov/docs/aids/aids_screen.html

Dimasi JA (2001). Risks in new drug development: approval success rates for investigational drugs, *Clinical Pharmacology and Therapeutics* 69:297–307.

Doll R and Hill AB (1950). Smoking and carcinoma of the lung (preliminary report). *Brit Med J* 117:39-48.

Eason CT, Bonner FW, Parke DV (1990). The importance of pharmacokinetic and receptor studies in drug safety evaluation. *Regulatory Toxicology and Pharmacology* 11:288-307.

ECEAE (European Coalition to End Animal Experiments) 7th Amendment to the Cosmetics Directive (2003/15/EC). [Accessed May 29 2007] Available at: http://www.eceae.org/english/cosmetics.html

ECVAM (European Centre for the Validation of Alternative Methods) [Accessed May 29 2007] Available at: http://ecvam.jrc.it/index.htm

Ekwall B. et al. (1998). MEIC evaluation of acute systemic toxicity: Part VI. The prediction of human toxicity by rodent LD50 values and results from 61 in-vitro methods. *ATLA* 26:617-58.

Ekwall B (1999). Overview of the final MEIC results: II. The in vitro/in vivo evaluation, including the selection of a practical battery of cell tests for prediction of acute lethal blood concentrations in humans. *Toxicology in Vitro* 13:665-73.

EMEA (2003). Position Paper on the Nonclinical Safety Studies to Support Clinical Trials with a Single Microdose, CPMP/SWP2599/02. [Accessed May 29 2007] Available at: www.emea.eu.int/pdfs/human/swp/259902en.pdf

Fauna Foundation (*a*), "Billy Jo History." [Accessed May 29 2007] Available at: http://www.faunafoundation.org/ff/english/sanct/chimps/billyjo.html

Fauna Foundation (*b*), "Chimpanzees in the biomedical industry." [Accessed May 29 2007] Available at: http://www.faunafoundation.org/ff/english/sanct/chimps/biomedical.html

Fishbein EA (2001). What price mice? *Journal of the American Medical Association* 235:939-41.

Gad SC (1990). Model selection in toxicology: principles and practice. *Journal of the American College of Toxicology* 9:291-302.

Gad SC (2000). Alternatives to in vivo studies in toxicology, in General and Applied Toxicology, Vol. 1, 2nd ed, Ballantyne B, Marrs TC and Syversen T (editors) (London: Nature):401–24.

Genschow E, Scholz G, Brown N, et al. (2000). Development of prediction models for three *in vitro* embryotoxicity tests in an ECVAM validation study. *In Vitro & Molecular Toxicology* 13:51-65.

Graham DJ, Campen D, Hui R, et al. (2005). Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclo-oxygenase 2 selective and non-selective non-steroidal anti-inflammatory drugs: nested case-control study. *Lancet* 365:475-81.

Gray JE, Weaver RN, Bollert JA, Feenstra ES (1972). The oral toxicity of clindamycin in laboratory animals. *Toxicology and Applied Pharmacology* 21:516-31.

Gruber FP and Hartung T (2004). Alternatives to animal experimentation in basic research. *ALTEX* 21 (Suppl. 1/04):3-31.

Harding, A (2004). More compounds failing phase I. FDA chief warns that high drug attrition rate is pushing up the cost of drug development. *The Scientist* 5(1): (6 August).

Harrison KA, Dalrymple GV, Baranowska-Kortylewicz J, et al. (1996). Radiolabeled iododeoxyuridine: safety evaluation. *Journal of Nuclear Medicine* 37 (Suppl 4):S13-6.

Hartung T, Bremer S, Casati S, et al. (2003). ECVAM's response to the changing political environment for alternatives: consequences of the European union chemicals and cosmetics policies. *ATLA* 31:473–81.

Haseman, J.K., Ney, E,. Nyska, A. and Rao, G.N (2003). Effect of diet and animal care/housing protocols on body weight, survival, tumor incidences, and nephropathy severity of F344 rats in chronic studies. *Toxicologic Pathology* 31:674-81.

Hjelle JJ and Grauer GF (1986). Acetaminophen-induced toxicosis in dogs and cats. *Journal of the American Veterinary Medical Association* 188:742-6.

Hoffmann S and Hartung T (2006). Toward an evidence-based toxicology. *Human & Experimental Toxicology* 25:497-513.

Home Office (2005). Examples of Reduction, Refinement and Replacement (3Rs). [Accessed May 29 2007] Available at: http://scienceandresearch.homeoffice.gov.uk/animal-research/publications-and-reference/publications/the-3rs/examples3rs.pdf?view=Binary

Home Office (2006). Statistics of Scientific Procedures on Living Animals in Great Britain 2005. Publication number CM6877. HMSO, London. [Accessed May 29 2007] Available at: http://www.homeoffice.gov.uk/rds/pdfs06/spanimals05.pdf

HSUS (Humane Society of the United States) (2001). Poll Shows Americans Disapprove of Animal Research when it Causes the Animals to Suffer. November 14. [Accessed May 29 2007] Available at: http://www.hsus.org/press_and_publications/press_releases/poll_shows_americans_disapprove_of_animal_research_when_it_causes_the_animals_to_suffer.html

HSUS (2002). [Accessed May 29 2007] Available at: http://www.hsus.org/animals_in_research/animals_in_research_news/great_britain_releases_animal_research_statistics_for_2002.html

HSUS (2006). Class B dealer down and out. [Accessed May 29 2007] Available at: http://www.hsus.org/animals_in_research/general_information_on_animal_research/class_b_dealer_down_and_out.html

HSUS (undated). [Accessed May 29 2007] Available at: http://www.hsus.org/animals_in_research/pain_distress/hsus_letter_on_underreporting_of_pain_and_distress/

Huggins J (2003a). Alternatives to skin corrosion/irritation testing in animals. *ALTEX* 20 (Suppl 1):19-25.

Huggins J (2003b). Alternatives to developmental/reproductive toxicity testing in animals. *ALTEX* 20 (Suppl 1):32-41.

Hughes TR, Marton MJ, Jones AR, et al. (2000). Functional discovery via a compendium of expression profiles. *Cell* 102:109-26.

Hurst JL, Barnard CJ, Tolladay U, Nevison CM, West CD (1999). Housing and welfare in laboratory rats: effects of cage stocking density and behavioural predictors of welfare. *Animal Behaviour* 58:563-86.

ICCVAM (The Interagency Coordinating Committee on the Validation of Alternative Methods) (2005). "Test Method Evaluations." [Accessed May 29 2007] Available at: http://iccvam.niehs.nih.gov/methods/methods.htm

Jimenez B, Eljarrat E, Hernandez LM, Rivera J, Gonzalez MJ (1996). Polychlorinated dibenzo-p-dioxins and dibenzofurans in soils near a clinical waste incinerator in Madrid, Spain. Chemometric comparison with other pollution sources and soils. *Chemosphere* 32:1327-48.

Johnston MI (2000). The role of nonhuman primate models in AIDS vaccine development. *Molecular Medicine Today* 6:267-70.

Jukes N, Chiuia M (2003). *From Guinea Pig to Computer Mouse: Alternative methods for a progressive, humane education (2nd edition).* InterNICHE. ISBN 1-904422-00-4.

Kerkvliet GJ (1990). Drug discovery screen adapts to changes. *Journal of the National Cancer Institute* 82:1087-8.

Khera KS (1976). Teratogenicity studies with methotrexate, aminopterin, and acetylsalicylic acid in domestic cats. *Teratology* 14:21-7.

Klausner M, Sheasgreen J, Kubilus J, Hayden P (2005). Long term reproducibility of epiocular™, a three-dimensional tissue culture model of the human corneal epithelium. *The Toxicologist* 84(1), Abstract # 2002, 409.

Knight A, Bailey J, Balcombe J (2005). Animal carcinogenicity studies: poor human predictivity. *Proceedings, 5th World Congress on Alternatives & Animal Use in the Life sciences, Berlin, August 21–25, 2005*.

Kola I and Landis J (2004). Can the pharmaceutical industry reduce attrition rates? *Nature Reviews Drug Discovery* 3:711–5.

Koppanyi T and Avery MA (1966). Species differences and the clinical trial of new drugs: a review. *Clinical Pharmacology & Therapeutics* 7:250-70.

Krasula RW, Pernet AG (1991). Comparison of organ-specific toxicity of temafloxacin in animals and humans. *American Journal of Medicine* 91:S38-41.

Krewski D, Burnett R, Goldberg M, et al. (2003). Overview of the reanalysis of the Harvard six cities study and American Cancer Society study of particulate air pollution and mortality. *Journal of Toxicology and Environmental Health Part A* 66:1507-52.

Lappin G, Kuhnz W, Jochemsen R, et al. (2006). Use of microdosing to predict pharmacokinetics at the therapeutic dose: experience with 5 drugs. *Clinical Pharmacology & Therapeutics* 80:203-15.

Lazarou J, Pomeranz BH, Corey PN (1998). Incidence of adverse drug reactions in Hospitalized patients: A meta-analysis of prospective studies. *Journal of the American Medical Association* 279:1200–05.

Liebsch M, Traue D, Barrabas C, et al. (1999). Prevalidation of the EpiDerm™ skin corrosivity test. *ATLA* 27:350.

Little CC (1961). Some phases of the problem of smoking and lung cancer. *New England Journal of Medicine* 264:1241-5.

Lumley CE and Walker SR (1990). In: Animal Toxicity Studies: Their Relevance for Man, Quay, Lancaster, UK:73.

Macleod MR, O'Collins T, Howells DW, Donnan GA (2004). Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke* 35:1203-8.

Macleod M (2005). What can systematic review and meta-analysis tell us about the experimental data supporting stroke drug development? *International Journal of Neuroprotection and Neuroregeneration* 1:201.

Mandal PK (2005). Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology B* 175:221-30.

Manier JW, Chang WW, KirchnerJP, Beltaos E (1982). Hepatotoxicity associate with ticrynafen - a uricosuric diuretic. *American Journal of Gastroenterology* 77:401-4.

Marks MI (1975). Evaluation of four antiviral agents in the treatment of herpes simplex encephalitis in a rat model. *Journal of Infectious Diseases* 131:11-6.

Marx U, Embleton MJ, Fisher R, et al. (1997). Monoclonal antibody production: the report and recommendations of ECVAM Workshop 23. *ATLA* 25:121-37.

Maynard R (2004). Key airborne pollutants – the impact on health. *Science of the Total Environment* 334-5:9-13.

McColl JD (1967). Drug toxicity in the animal fetus. *Applied Therapeutics* 9:915-7.

MEIC – Multicentre Evaluation of *In Vitro* Cytotoxicity. Summary available at:
http://www.cctoxconsulting.a.se/meic.htm
[Accessed June 26 2007]

Millen JW (1962). Thalidomide and limb deformities. *Lancet* 2:599-600.

Mulder P, Richard V, Thuillez C (1998). Different effects of calcium antagonists in a rat model of heart failure. *Cardiology* 89(suppl 1):33-7.

National Assembly (CNRS, France), Report 3291: From mad cow to scape cow; vol 2 (1996). [Accessed May 29 2007] Available at:
http://www.assemblee-nationale.fr/11/rapports/r3291-01-x.asp
[Report in French]

National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group (1995). Tissue plasminogen activator for acute ischemic stroke. *New England Journal of Medicine* 333:1581-7.

National Research Council. (NRC) (1992). Pain and Distress in Laboratory Animals. Washington DC. National Academy Press. Table 4-3 (page 40).

National Research Council (NRC) (1996). *Guide for the Care and Use of Laboratory Animals, 7th Edition*. National Academy Press, Washington, DC.

National Toxicology Program (NTP) October 2006. Specifications for the conduct of studies to evaluate the toxic and carcinogenic potential of chemical, biological and physical agents. [Accessed May 29 2007] Available at:
http://ntp.niehs.nih.gov/files/SPECIFICATIONS_2006Oct.pdf

National Toxicology Program (NTP, 2006). NIEHS Fact Sheet #3. [Accessed May 29 2007] Available at:
http://www.niehs.nih.gov/oc/factsheets/fsntp.htm

O'Connor AM (1997). Barriers to regulatory acceptance. In van Zutphen LFM, Balls M, eds) *Animal Alternatives, Welfare and Ethics*. Amsterdam, The Netherlands: Elsevier Science B.V.:1173-6.

OECD (Organisation for Economic Co-operation and Development) (2000). Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation (ENV/JM/MOMO).

OECD (2001). Test Guideline 401 will be deleted: A Major Step in Animal Welfare: OECD Reaches Agreement on the Abolishment of the LD50 Acute Toxicity Test. [Accessed May 29 2007] Available at:

http://www.oecd.org/documentprint/0,2744,en_2649_34377_2752116_1_1_1_1,00.html

Oh J-E, Choi S-D, Lee S-J, Chang Y-S (2006). Influence of a municipal solid waste incinerator on ambient air and soil PCDD/Fs levels. *Chemosphere* 64:579-87.

OLPA (Office of Legislative Policy and Analysis) (2005). Pet Safety and Protection Act of 2005: S. 451. [Accessed May 29 2007] Available at: http://olpa.od.nih.gov/legislation/109/pendinglegislation/petsafety.asp

Olsson AS, Dahlborn K (2002). Improving housing conditions for laboratory mice: a review of 'environmental enrichment' *Laboratory Animals* 36:243-70.

O'Shea D (2000). Johns Hopkins enters suit over lab animal regulations. Press Release, 22 September. Johns Hopkins University, Baltimore.

OTA (Office of Technology Assessment), U.S. Congress (1986). *Alternatives to Animal Use in Research, Testing and Education.* Washington, D.C.: U.S. Government Printing Office, OTA-BA-273.

Perel P, Roberts I, Sena E, et al. (2006). [Accessed May 29 2007] Available at: http://www.pcpoh.bham.ac.uk/publichealth/nccrm/PDFs%20and%20documents/Publications/JH18_Final_Report_May_2006.pdf

Pirmohamed M, James S, Meakin S, et al. (2004). Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. *BMJ* 329(7456):15-9.

Plous S (undated) Opinion Research on Animal Experimentation: Areas of Support and Concern. [Accessed May 29 2007] Available at: http://altweb.jhsph.edu/meetings/pain/plous.htm

Pomati F, Castiglioni S, Zuccato E, et al. (2006). Effects of a complex mixture of therapeutic drugs at environmental levels on human embryonic cells. *Environmental Science & Technology* 40:2442-7.

Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I (2004). Where is the evidence that animal research benefits humans? *BMJ* 328:514-7.

Project R&R: Release and Restitution for Chimpanzees in U.S. Laboratories. [Accessed September 21, 2007] Available at http://www.releasechimps.org/2006/06/20/poll-reveals-americans-agree-chimpanzees-in-laboratories-for-more-than-10-years-should-be-retired.

Rai AJ, Zhang Z, Rosenzweig J, et al. (2002). Proteomic approaches to tumor marker discovery. *Arch Pathol Lab Med* 126:1518–26.

Rappersberger K, Komar M, Ebelin ME, et al. (2002). Pimecrolimus identifies a common genomic anti-inflammatory profile, is clinically highly effective in psoriasis and is well tolerated. *J Invest Dermatol* 119:876–87.

Roberts, R.A., Soames, A.R., James, N.H., et al. (1995) Dosing-induced stress causes hepatocyte apoptosis in rats primed by the rodent nongenotoxic hepatocarcingen cyproterone acetate. *Toxicol. Appl. Pharmacol*. 135:192-9.

Robertson RT, Allen HL, Bokelman DL (1979). Aspirin: teratogenic evaluation in the dog. *Teratology* 20:313-20.

Roe FJC (1993). Influence of animal species, strain, age, hormonal, and nutritional status, in Experimental Toxicology, The Basic Issues, 2nd Edition, Anderson D and Conning D (Editors) (Cambridge: The Royal Society of Chemistry):23–34.

Rosenberg SA, Yang JC, Restifo NP (2004). Cancer immunotherapy: moving beyond current vaccines. *Nature Medicine* 10:909-15.

Ross-Degnan D, Soumerai SB, Fortess EE, Gurwitz JH (1993). Examining product risk in context. Market withdrawal of zomepirac as a case study. *Journal of the American Medical Association* 270:1937-42.

Rowat SC (1999). Incinerator toxic emissions: a brief summary of human health effects with a note on regulatory control. *Medical Hypotheses* 52:389-96.

Sanders DD and Stephens TD (1991). Review of drug-induced limb defects in mammals. *Teratology* 44:335-54.

Sandusky C, Even M, Stoick K, Sandler J (2006). Strategies to reduce animal testing in US EPA's HPV program. *ALTEX* 23 (special issue):117-9.

Savides MC, Oehme FW, Nash SL, Leipold HW (1984). The toxicity and biotransformation of single doses of acetaminophen in dogs and cats. *Toxicology and Applied Pharmacology* 74:26-34.

Scherf U, Ross DT, Waltham M, et al. (2000). A gene expression database for the molecular pharmacology of cancer. *Nature Genetics* 24:236-44.

Schmidt CW (2000). Assessing assays. *Environmental Health Perspectives* 110:A248-51.

Schneierson SS, Perlman E (1956). Toxicity of penicillin for the Syrian hamster. *Proceedings of the Society for Experimental Biology and Medicine* 91:229-230.

Segura-Munoz SI, Takayanagui AMM, Trevilato TMB, Santos CB, Hering SE (2004). Trace metal distribution in surface soil in the area of a municipal solid waste landfill and a medical waste incinerator. *Bulletin of Environmental Contamination and Toxicology* 72:157-64.

Silverman E (2005). Monkey tests at heart of Merck case. *Newark Star Ledger* July 14.

Spriet-Pourra C, Auriche M (1994). Drug Withdrawal from Sale, 2nd edition, Scrip Reports, PJB Publications, New York, USA.

Steiner G, Suter L, Boess F, et al. (2004). Discriminating different classes of toxicants by transcript profiling. *Environ Health Perspect Toxicogenomics* 112:1236-48.

Stephens ML (2000). An Overview of Animal Testing Issues. [Accessed May 29 2007] Available at: http://www.hsus.org/animals_in_research/animal_testing/an_overview_of_animal_testing_issues/

Stephens ML, Conlee K, Alvino G and Rowan AN (2002). Possibilities for refinement and reduction: future improvements within regulatory testing *ILAR J* 43:S74–9.

Tatchell P (2004). Why animal research is bad science. *New Statesman* August 9.

Thomas RS, Rank DR, Penn SG, et al. (2001). Identification of toxicologically predictive gene sets using cDNA microarrays. *Molecular Pharmacology* 60:1189-94.

Thompson LJ, Ebel JG, Manzell KL, Rutzke M, Gutenmann WH, Lisk DJ (1995). Analytical survey of elements in veterinary college incinerator ashes. *Chemosphere* 30:807-11.

TNS Healthcare (2004) (www.tns-global.com). Poll results available online at: http://www.curedisease.net/news/040903.shtml [accessed July 18th, 2006]

Topol EJ (2004). Failing the public health – rofecoxib, Merck, and the FDA. *New England Journal of Medicine* 351:1707-9.

USDA's Office of Inspector General (OIG), Oct. 20, 2005. APHIS Animal Care Program Inspection and Enforcement Activities. Highlights in "AWI Quarterly," publication of the Animal Welfare Institute, Winter 2006: 55(1). [Accessed May 29 2007] Available at: http://www.animalwelfare.com/pubs/Quarterly/06-55-01/06_55_1p9.htm

USGS. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams. June 2002.

Van Driel K and Talling JC (2005). Familiarity increases consistency in animal tests. *Behavioural Brain Research* 159:243-5.

Venning GR (1983). Identification of adverse reactions to new drugs. I: What have been the important adverse reactions since thalidomide? *British Medical Journal* 286:199-202.

Villar D, Buck WB, Gonzalez JM (1998). Ibuprofen, aspirin and acetaminophen toxicosis and treatment in dogs and cats. *Veterinary and Human Toxicology* 40:156-62.

von Keutz E and Schluter G (1998). Preclinical safety evaluation of cerivastatin, a novel HMG-CoA reductase inhibitor. *American Journal of Cardiology* 82:J11-17.

Wallenstein L and Snyder J (1952). Neurotoxic reaction to chloromycetin. *Annals of Internal Medicine* 36:1526-8.

Ward A (1990). Consumers at odds with animal testing. *Advertising Age* February 26:61 (s2).

Wiebers DO, Adams HP, Whisnant JP (1990). Animal models of stroke: are they relevant to human disease? *Stroke* 21:1-3.

Wieland S, Thimme R, Purcell RH, Chisari FV (2004). Genomic analysis of the host response to hepatitis B virus infection. *Proceedings of the National Academy of Science* 101:6669-74.

Wilson JG, Gavan JA (1967). Congenital malformations in nonhuman primates: spontaneous and experimentally induced. *Anatomical Record* 158:99-109.

Wilson JG, Ritter EJ, Scott WJ, Fradkin R (1977). Comparative distribution and embryotoxicity of acetylsalicylic acid in pregnant rats and rhesus monkeys. *Toxicology and Applied Pharmacology* 41:67-78.

Würbel H (2002). Behavioral phenotyping enhanced – beyond (environmental) standardization. *Genes, Brain and Behavior* 1:3-8.

Wynder DL, Graham EA (1950). Tobacco smoking as a possible etiologic factor in bronchiogenic cancer. *Journal of the American Medical Association* 143:329-36.

APPENDIX A: Non-Animal Methods in Research and Education

(I) Replacement Methods in Animal Research

In contrast to current regulatory practices that in many cases require the use of animals for drug and device approvals, there are no regulations in basic science research requiring scientists to perform animal procedures. Nevertheless, the number of animals used in basic research has been increasing, due largely to increased use of transgenic animals (Carbone, 2004). Gruber and Hartung (2004) put forward a number of reasons why change has been slow, and present several examples of human tissue and organ methods that can be successfully adopted in many research programs currently using animals. They suggest that tradition and inertia dictate current animal use and that there is a perceived lack of will to shift the paradigm in such a way that animal experiments are obviated. In addition, the slow adoption of non-animal methods is sustained by scientists who deny their adequacy in the face of evidence to the contrary, and authorities that, having no overview of existing alternatives, grant permission for the animal procedures.

There is much debate about the availability and suitability of replacement methods in some areas of medical research. Interested parties argue over the merits of animal-based methods that characterize the traditional standards of practice in some research fields, and the virtues of in vitro, in silico, and human-based methods that may potentially replace those methods. In such circumstances the responsibility of scientists using animal-based approaches, their regulators, oversight and ethics committees, and funding sources includes not only valid scientific and ethical justification for these practices but also proactive efforts to identify and implement methods that replace, reduce, or refine such animal uses.

(II) Examples of Successful Replacement Methods in Medical Research

- A strain of insulin-producing pancreas cells has been developed that are able to survive in culture for long periods – saving hundreds of animals and accelerating diabetes research.
- The use of mice and rats in sleeping sickness research has been superseded by developing a method of growing the parasite in vitro.
- TMS (transcranial magnetic stimulation) is now used to study the human brain. Non-invasive investigations in human volunteers are now possible instead of highly invasive brain damaging experiments on monkeys.
- Cell cultures replaced the use of thousands of mice over five years in researching infant brain damage and multiple sclerosis.
- Magnetoencephalography (MEG) can noninvasively detect activity in the human brain, enabling relevant research into vision, hearing, epilepsy, brain injury, pain and neurological illness in humans, in place of experiments on cats and monkeys with recording electrodes bolted into their skulls.
- Identification of areas of the brain involved in processing pain is now being carried out using a laser pain stimulator that can be used alongside brain imaging techniques such as EEG, PET and fMRI. This enables the development of more effective human pain control therapies than by using rodents, cats, and monkeys.
- Cells of the human lens are now used in place of animals in cataract research.
- Cultured human cartilage tissue is now used for rheumatism research in place of painful animal tests, in which chemicals or bacteria are injected into the joints and

paws of rabbits, rats and mice. They have been instrumental in the discovery of chemical and structural changes that accompany rheumatism, and in the discovery of how anti-rheumatic drugs work.

- Differences in blood flow that can cause eye and kidney problems have been identified between diabetics and healthy volunteers, using laser Doppler perfusion imaging. This has resulted in directly relevant data, without the use of animal models of dubious relevance.
- Safe methods of studying the arteries of young children have been developed to explain why sufferers of heart disease and stroke are more likely to have been small at birth. Previously, scientists relied on extrapolating information from artificial animal models.
- Alzheimer's disease is incurable and its causes unknown, despite experiments on monkeys and genetically engineered mice. Groundbreaking research utilizing human brain tissue has revealed risk factors involved in its development, and two types of virus associated with the brains of Alzheimer's sufferers.
- Animal models in AIDS research are infamously poor. Cell culture work has elucidated many aspects of the viral life cycle in humans, and helped enormously in the development of new drugs to combat the disease.
- Tissue banks are expanding in number and size to provide a reliable supply of ethically sourced human tissue for biomedical research, and to encourage approaches that use human tissue as an alternative to animals.
- Novel analytical techniques are now being used to identify disease-causing microbes in place of tests using rabbits and guinea pigs. Pulses of laser light generate a unique "fingerprint" for each type of microbe that can be used to identify different forms of bacteria, previously only distinguishable by animal tests that involved inducing abscesses on guinea pigs' legs.
- Clinical trials are now underway to see if citrus flavonoids can help treat patients with the most malignant form of brain tumor, following cell culture studies of human brain cells. Traditionally, tumors are implanted into rats, but they differ considerably from human brain tumors in the way they grow and spread.

All the above examples are taken from the archives of the Dr Hadwen Trust (www.drhadwentrust.org.uk), specifically
http://www.drhadwentrust.f2s.com/J_FS-SS_SuccessStories.html and
http://www.drhadwentrust.f2s.com/J_FS-CC_CellCultureSuccess.html
and from the Lord Dowding Fund (www.navs.org.uk/research/49/51/287).

(All accessed June 5, 2007)

(III) Alternatives in Health Science and Medical Education

Harmful and consumptive animal use has historically played an integral role in all levels of health sciences education. Scientific, medical and veterinary education in the US has relied on harming and killing healthy animals to teach biology, anatomy, physiology, pharmacology, anesthesiology, critical care, emergency medicine, diagnostic and therapeutic procedures, surgery and trauma skills, and other principles and procedures.

However, since the late 1970s student conscientious objection to harming and killing animals has risen sharply. Now more than half of US veterinary schools have no terminal surgeries and almost all these veterinary schools permit the use of alternatives to harmful

procedures on animals. A similar situation has occurred in human medical education, and now 90 percent of US allopathic and osteopathic medical schools have eliminated live animal labs from their curricula.

Concerns about harming animals in order to learn to be healers stem from ethical concerns regarding animal treatment, becoming desensitized to animal suffering, and the negative emotional response to participating in procedures where animals are harmed or killed. Consequently, the use of humane alternatives has grown steadily and significantly.

Of all areas in which living animals are used in laboratory procedures, the use of animals in education provides perhaps the least defensible argument for their use. Instructional use of animals is typically repetitive, is done to impart existing knowledge, and is eminently replaceable with equivalent or superior educational methods.

Proof lies in the numerous internet databases and other resources which list thousands of validated educational alternatives for all levels of education.[16] For example, NORINA (Norwegian Reference Center for Laboratory Animal Science and Alternatives Audiovisuals Database), EURCA (European Resource Center for Alternatives to Animal Use in Higher Education), and Alternatives in Education Database by the Association of Veterinarians for Animal Rights, are a few commonly used alternatives databases. Additionally, the 2nd edition of the International Network for Humane Education (InterNICHE) book *From Guinea Pig to Computer Mouse: Alternative Methods for a Progressive, Humane Education* lists more than 500 alternative products covering a broad range of life and health science disciplines. These disciplines include anesthesia, critical care, anatomy, biochemistry, cell biology, clinical and surgery skills, embryology, developmental biology, histology, pathology, pharmacology, and physiology (Jukes and Chiuia, 2003).

Numerous types of validated non-animal methods are available in medical education. For example, inanimate training models are less expensive than live animals, and have been designed to help students learn numerous procedures such as intubation, injections, suturing, CPR, blood collection, intravenous catheter placement, surgery skills and intravascular procedures, among others.

Most veterinary and medical schools include computer-aided training programs in their curricula. For example, students at the University of California School of Veterinary Medicine can review graphics, videos, case studies, and other information specific to a clinical case or for general procedures. Interactive computer programs, computer-generated models, and virtual reality programs replace animal use for many basic science and clinical skills applications, and also in pre-college science classrooms.

Imaging methods such as standard radiology, ultrasonography, computed tomography, and magnetic resonance imaging are routinely used in basic science and clinical medical education. Lifelike programmable human simulators have revolutionized medical education in many areas, contributing importantly to the rapid decline in live animal laboratories and expanding the capabilities of preclinical hands-on training. Willed body or ethically sourced donation programs exist, in which people donate their own bodies, or those of companion or farm animals, for use in training and education.

[16] http://www.learningwithoutkilling.info/links_and_lists/databases.htm

Many of these alternative educational methods allow students to employ repetition and iterative learning, self-paced and offsite education, and immediate correction of errors to gain confidence and achieve specific training goals – advantages documented to enhance learning, but not provided by live animal laboratories.

At least 30 studies in biomedical and educational literatures covering virtually all applicable educational levels and biomedical disciplines have examined the ability of humane alternatives to impart basic science knowledge and clinical or surgical skills (Balcombe et al., 2000). More than one-third of these studies (11/30) demonstrated that students achieved superior learning outcomes, or achieved equivalent results more quickly, allowing time for additional learning. Nearly all studies (28/30) demonstrated at least equivalent educational efficacy. Only two of the 30 studies demonstrated reduced educational efficacy, and the design of one of those studies has been criticized.

Twenty-two other non-animal teaching studies are also listed by Balcombe, which demonstrated time and cost savings associated with humane teaching methods. The costs of acquiring and using most humane alternatives, which are reusable or available through subscriptions or site licenses, are much lower than the costs associated with obtaining, maintaining, and disposing of animals (Balcombe, 2000). Increased repeatability and flexibility of use, customization of laboratory experiences, facilitation of interactive, autonomous and life-long learning, improved attitudes toward computers and alternatives to animal use, and increased perception of computer literacy were other benefits.

Despite the clear advantages of using humane alternatives in biomedical education, harmful or lethal animal use remains common in pre-college and university level health sciences education within the US. However, the trend is away from such animal use, and it is reasonable to conclude that replacement of animal use will accelerate as knowledge of validated equivalent or superior alternatives becomes more widespread.

APPENDIX B: Excerpts of US and European Laws with Relevance for Replacement Methods

US Law

I. Section 495 of the **Health Research Extension Act (HREA)** of 1985 states in relevant part:

(a) The Secretary, acting through the Director of NIH, shall establish guidelines for the following:
(1)The proper care of animals to be used in biomedical and behavioral research.
(2)The proper treatment of animals while being used in such research. ...
(3)The organization and operation of animal care committees in accordance with subsection (b).
(4)Each animal care committee of a research entity shall—
(A) review the care and treatment of animals in all animal study areas and facilities of the research entity at least semiannually to evaluate compliance with applicable guidelines established under subsection (a) for appropriate animal care and treatment; (C) for each review conducted under subparagraph (A), file with the Director of NIH at least annually (i) a certification that the review has been conducted, and (ii) reports of any violations of

guidelines established under subsection (a) or assurances required under paragraph (1) which were observed in such review and which have continued after notice by the committee to the research entity involved of the violation. Reports filed under subparagraph (C) shall include any minority views filed by members of the committee.
(c) The Director of NIH shall require each applicant for a grant, contract, or cooperative agreement involving research on animals which is administered by the National Institutes of Health ... to include ...
(1) Assurances satisfactory to the Director of NIH that—
(A) the applicant ... has an animal care committee which meets the requirements of subsection (b); and
(B) scientists, animal technicians, and other personnel involved with animal care, treatment, and use by the applicant have available to them instruction or training in the humane practice of animal maintenance and experimentation, and the concept, availability, and use of research or testing methods that limit the use of animals or limit animal distress; and
(2) a statement of the reasons for the use of animals in the research to be conducted with funds provided under such grant or contract. ...
(d) If the Director of NIH determines that
(1) the conditions of animal care, treatment, or use in an entity which is receiving a grant, contract, or cooperative agreement involving research on animals under this title do not meet applicable guidelines established under subsection (a);
(2) the entity has been notified by the Director of NIH of such determination and has been given a reasonable opportunity to take corrective action; and
(3) no action has been taken by the entity to correct such conditions; the Director of NIH shall suspend or revoke such grant or contract under such conditions as the Director determines appropriate. ...

II. Section 205 of the National Institutes of Health Revitalization Act (NIHRA) of 1993 states in relevant part:

(a) The Director of NIH, after consultation with the committee established under subsection (e), shall prepare a plan–
(1) for the National Institutes of Health to conduct or support research into–
(A) methods of biomedical research and experimentation that do not require the use of animals;
(B) methods of such research and experimentation that reduce the number of animals used in such research;
(C) methods of such research and experimentation that produce less pain and distress in such animals; and
(D) methods of such research and experimentation that involve the use of marine life (other than marine mammals);
2) for establishing the validity and reliability of the method(s) described in paragraph (1);
(3) for encouraging the acceptance by the scientific community of such methods that have been found to be valid and reliable; and
(4) for training scientists in the use of such methods that have been found to be valid and reliable.
(b) Not later than October 1, 1993 the Director of NIH shall submit to the Committee on Energy and Commerce of the House of Representative(s), and to the Committee on Labor and Human Resources of the Senate, the plan required in subsection (a) and

shall begin implementation of the plan.
(c) The Director of NIH shall periodically review, and as appropriate, make revisions in the plan required under subsection (a). A description of any revision made in the plan shall be included in the first biennial report under section 403 that is submitted after the revision is made.
(d) The Director of NIH shall take such actions as may be appropriate to convey to scientists and others who use animals in biomedical or behavioral research or experimentation information respecting the methods found to be valid and reliable under sub-section (a)(2).
(e)(1) The Director of NIH shall establish within the National Institutes of Health a committee to be known as the Interagency Coordinating Committee on the Use of Animals in Research (in this subsection referred to as the 'Committee').
(2) The Committee shall provide advice to the Director of NIH on the preparation of the plan required in subsection (a).
(3) The Committee shall be composed of–
(A) the Directors of each of the national research institutes and the director of the Center for Research Resources (or the designees of such Directors); and
(B) representatives of the Environmental Protection Agency, the Food and Drug Administration, the Consumer Product Safety Commission, the National Science Foundation, and such additional agencies as the Director of NIH determines to be appropriate, which representatives shall include not less than one veterinarian with expertise in laboratory-animal medicine.

III. In response to the NIHRA, the *Plan for the Use of Animals in Research* addresses the act's objectives by establishing the need to:

- Evaluate the effectiveness of previous Trans-NIH Program announcements to encourage the submission of applications for investigations into methods that do not require animals, reduce the numbers of animals, or lessen pain and distress in animals. Use of this evaluation to determine new areas of research that meet the objectives of the legislation. This activity explores the use of lower organisms, cultured tissues and cells, and mathematical and computer simulations as models for biomedical and behavioral research.
- Support for ongoing NIH research projects and consider issuing new solicitation for projects that use cell cultures or other in vitro systems as models for screening prior to animal testing.
- Continue NIH support for resource centers that produce and supply critical biomaterials to researchers as models for biomedical and behavioral research such as cloned genes/vectors, DNA probes, chromosomes; stably transfected cell lines; micro-organisms .... Promote the availability of and disseminate these resource materials to researchers seeking assistance and collaboration in health research.
- Establish an Advisory Panel composed of experts from Federal agencies to review and evaluate new technologies that meet the objectives of this legislation.
- Incorporate training in new and diverse technologies into interdisciplinary training programs (e.g., mathematics and computer sciences) in accord with the objectives of this legislation. These training programs can be incorporated into NIH training grants awarded to eligible institutions and into predoctoral and postdoctoral fellowships to prepare future scientists for careers in biomedical and behavioral research.

- Promote the training of scientists who use research animals in the use of methods that have been found to be valid and reliable in accord with the objectives of this legislation; and promote the training of animal researchers and members of animal care and use committees on the importance of power and sample size planning in research design.

IV. Department of Defense Directive AR 40–33/SECNAVINST 3900.38C/AFMAN 40–401(I)/DARPAINST 18/USUHSINST 3203: "The Care and Use of Laboratory Animals in DoD Programs," effective March 16, 2005, states in relevant part:

... *5 b. Other methods.* Alternative methods to the use of animals must be considered and used if such alternatives produce scientifically valid or equivalent results to attain the research, education, training, and testing objectives…

DEFINITIONS

… E2.1.1. Animal.
Any dog, cat, non-human primate, guinea pig, hamster, rabbit or any other live vertebrate animal, which is being used or is intended for use for research, training, testing, or experimentation purposes. For this Directive, it includes birds, rats of the
genus Rattus and mice of the genus Mus bred for use in research, training, testing or experimentation purposes. …
E2.1.5. Alternatives.
Any system or method that covers one or more of the following: replacing or reducing the number of laboratory animals required for an investigation by computer simulation, cell culture techniques, etc.; or, refining an existing procedure or technique to minimize the level of stress endured by the animal. …

V. Public Health Service Policy on Humane Care and Use of Laboratory Animals (as amended August 2002) states, in part:

1. "No activity involving animals may be conducted or supported by the PHS until the institution conducting the activity has provided a written Assurance acceptable to the PHS, setting forth compliance with this Policy. ...

"The Assurance shall fully describe the institution's program for the care and use of animals in PHS-conducted or supported activities. ... The program description must include the following:

... g. a synopsis of training or instruction in the humane practice of animal care and use, as well as training or instruction in research or testing methods that minimize the number of animals required to obtain valid results and minimize animal distress, offered to scientists, animal technicians, and other personnel involved in animal care, treatment, or use;

"Applications and proposals (competing and non-competing) for awards submitted to PHS that involve the care and use of animals shall contain the following information:

... b. rationale for involving animals, and for the appropriateness of the species and numbers used;"

VI. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (1985):

"The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered."

VII. California Civil Code section 1834.8: Deposited/Abandoned Animals and Animal Testing states, in relevant part:

a) Manufacturers and contract testing facilities shall not use traditional animal test methods within this state for which an appropriate alternative test method has been scientifically validated and recommended by the Inter-Agency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) and adopted by the relevant federal agency or agencies or program within an agency responsible for regulating the specific product or activity for which the test is being conducted.
(b) Nothing in this section shall prohibit the use of any alternative non-animal test method for the testing of any product, product formulation, chemical, or ingredient that is not recommended by ICCVAM.
(c) Nothing in this section shall prohibit the use of animal tests to comply with requirements of state agencies. Nothing in this section shall prohibit the use of animal tests to comply with requirements of federal agencies when the federal agency has approved an alternative non-animal test pursuant to subdivision (a) and the federal agency staff concludes that the alternative non-animal test does not assure the health or safety of consumers.
... (e) This section shall not apply to any animal test performed for the purpose of medical research.

<u>European Law</u>

This section presents excerpts of European laws relevant to the petition.

I. EUROPEAN COUNCIL DIRECTIVE 86/609/EEC

COUNCIL DIRECTIVE of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC)
THE COUNCIL OF THE EUROPEAN COMMUNITIES,
Having regard to the Treaty establishing the European Economic Community
... HAS ADOPTED THIS DIRECTIVE:
... (d) 'experiment' means any use of an animal for experimental or other scientific purposes which may cause it pain, suffering, distress or lasting harm, including any course of action intended, or liable, to result in the birth of an animal in any such condition, but excluding the least painful methods accepted in modern practice (i.e. 'humane' methods) of killing or marking an animal; an experiment starts when an animal is first prepared for use and ends when no further observations are to be made for that experiment; the elimination of pain, suffering, distress or lasting harm by the successful use of anesthesia or analgesia or other methods does not place the use of an animal outside the scope of this definition. Non experimental, agricultural or clinical veterinary practices are excluded;

... Article 7
... 2. An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.
3. When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected.

II. European Ban on Animal Testing

In February 1992 the European Parliament signaled its commitment to sustained progress regarding alternatives to animals by voting to ban the marketing of cosmetics containing ingredients that have been tested on animals after January 1, 1998. This far-reaching legislation prohibited marketing in Europe of products tested on animals or which contain ingredients tested on animals, even if those products are manufactured outside Europe. Industrial chemicals are exempt from the ban (CAAT, undated).

The 1998 date was ultimately extended, and on February 27, 2003 the 7th amendment to the 1976 Cosmetics Directive was approved after negotiations between the European Parliament and the Council of Ministers. This new Directive introduces a detailed timeline for the phase-out of animal tests of cosmetics, summarized below:

- from September 2004, a ban on testing of finished products within the EU
- from September 2004, a ban on the marketing of cosmetic products and ingredients tested on animals outside the EU, where alternative tests, validated and adopted in the EU, exist
- from September 2009, a ban on animal testing of cosmetic ingredients within the EU
- from 2009, a ban on the marketing of cosmetic products and ingredients tested on animals for the majority of such tests, irrespective of the availability of non-animal tests, and
- from 2013, a ban on cosmetic products and ingredients tested using three additional animal tests (ECEAE 2004)

Case Study: EU 86/609/EEC

Passage of Directive 86/609/EEC in 1986 by the Council of Europe is widely acknowledged as a primary factor responsible for Europe's pre-eminence in the field of non-animal methods (as measured by fewer animals used, more test methods validated and approved, and stronger legislation). This document codifies the principles of the 3Rs, and plainly asserts the principle that it is scientifically and morally insupportable to harm animals when valid alternatives may be used instead. The current petition draws on two decades of experience with alternatives implementation in EU member states.

While it is impossible to attribute European advances in non-animal methods to any one piece of legislation, it is equally clear that EU Directive 86/609/EEC has had positive effects. In general, the most significant of these is to mandate non-animal alternatives into the political agenda in all EU countries. Because EU directives must be implemented through national legislation, all member states have had to grapple with and implement

strategies for the deployment of non-animal methods. Some of these countries (e.g., Hungary, Latvia, Lithuania, Poland, Slovakia and Slovenia) lacked any mention of alternatives in their pre-existing laws.

A significant European-wide development regarding alternatives to animal use was the establishment of ECVAM in 1991. ECVAM's primary duties are four-fold:

1) To coordinate the validation of alternative test methods at the European Union level
2) To act as a focal point for the exchange of information on the development of alternative test methods
3) To set up, maintain and manage a data base on alternative procedures, and
4) To promote dialogue between legislators, industries, biomedical scientists, consumer organizations and animal welfare groups, with a view to the development, validation and international recognition of alternative test methods

ECVAM has its own Scientific Advisory Committee (ESAC) with participation from all member states, as well as relevant industrial associations, academic toxicology experts, animal welfare organizations, and other European Commission services with interest in non-animal methods. ECVAM's activities are undertaken in collaboration with numerous laboratories and organizations in the EU member states and around the world.

Directive 86/609/EEC does not limit member nations from exceeding its requirements. For instance, in 1989 the Netherlands introduced a Code of Practice for the Production of Monoclonal Antibodies. Dutch researchers took notice, and the pace of adoption of in vitro alternatives increased. It soon became apparent that in vivo production of monoclonal antibodies was not justifiable, and the practice was prohibited. Similar actions have occurred in Germany, Sweden, Switzerland and the United Kingdom. By 1996, in vitro monoclonal antibody production was the method of choice in Europe (Anonymous, 1999). In addition to ECVAM, Europe has several national 3Rs centers, with domestic programs dedicated to advancing non-animal methods.

Among the more concrete advances in non-animal methods in the EU is in the reduction of animals used in toxicity tests. For example, in the 1970s the standard Lethal Dose 50 (LD50) test required about 20-40 animals per dose group, with five dose groups, and two species, a total of 200 to 300 animals. Today, about 40 animals are required in total, due to revised testing protocols. In 1997, 139,000 animals were subjected to LD50 tests in British labs, but in 1999 Great Britain announced it would no longer approve LD50 testing protocols.

Directive 86/609/EEC has also helped to spawn additional European legislation, most notably the European Union Cosmetics Directive, which was enacted in 1993 and took effect in 2000. This legislation bans the marketing in Europe of any cosmetics tested on animals after 1998, including products manufactured in the USA. Today, EU 86/609/EEC and the concept of alternatives use are accepted as standard practice by EU-based researchers and industry. It has become almost axiomatic that the use of available non-animal methods is preferable in the pursuit of best practices.

APPENDIX C: Scientific Support for the Petition

This petition is submitted with the support of the following organizations and individuals:

Organizations

Animal Protection Institute (API)
Antidote Europe
Association for the Acknowledgement of the Universal Rights of Animals(ARUDA)
Association of ARABEL
Association of Veterinarians for Animal Rights (AVAR)
British Union for the Abolition of Vivisection (BUAV)
Comitato Europeo Difesa Animali (CEDA)
Ecological Movement National One Man Nature Animals
EQUIVITA
Humane Society Legislative Fund (HSLF)
Humane Society of the United States (HSUS)
In Defense of Animals (IDA)
Invitro International
New England Anti-Vivisection Society (NEAVS)
Physicians Committee for Responsible Medicine (PCRM)

Individuals

Aysha Akhtar, MD, MPH, Neurologist, Senior Medical and Research Adviser (PCRM)

Richard Allan, DVM

Ciro Aurigemma, Psychologist

Jarrod Bailey, PhD, University of Newcastle Upon Tyne

Jonathan Balcombe, PhD, MS, Ethologist, Research Scientist (PCRM)

Maria Grazia Barbieri, National President, Centro Ricerca Cancro Senza Sprimentazione Animale

Neal D. Barnard, MD, President and Founder (PCRM)

Teri Barnato, MA, National Director (AVAR)

Marc Bekoff, PhD, Ethologist, Professor Emeritus of Ecology and Evolutionary Biology, University of Colorado at Boulder

Antonella Bellingeri

Dr. Stephanie Benner

Gerry Boss, MD, Internal Medicine Physician

Lisa M. Brothwell, PhD, Psychologist

Nedim C. Buyukmihci, VMD, Emeritus Professor of Veterinary Medicine, University of California and Emeritus Diplomate, American College of Veterinary Ophthalmologists

Luigi Campanella, Full Professor of Environmental and Cultural Heritage Chemistry, University of Rome La Sapienza

Theodora Capaldo, EdD, President (NEAVS)

Francesco Carlini, Medical Doctor

Dr. Chiara Cerilli

Randal Charlton, Founder and Former CEO, Asterand plc

Holly Cheever, DVM, Vice President (AVAR)

Pietrina Cheesa, President (ARUDA)

Cecilia Clemedson, PhD

Murry J. Cohen, MD

Mina Connor, DVM

Professor Carlo Consiglio

Marjorie Cramer, MD, FACS

Lee Cranberg, MD

Paul F. Cunningham, PhD, Professor of Psychology, Rivier College

Dr. & Mrs. Joseph P. Curley, MD, Berkshire Medical Center

Catherine Dell'Orto, DVM, MPH

W. Jean Dodds, DVM, Hematologist/Immunologist, President (HEMOPET)

Raffaella Fabbri

Ebe Dalle Fabbriche, President (Ecological Movement National One Man Nature Animals)

Bruce Max Feldmann, DVM, University of California-Berkeley

Ilaria Ferri, Animalisti Italiani, Director and Minister Advisor, Ministero dell'Ambiente e Tutela del Territorio e del Mare

Jill Fetell, MD, Tarrytown Functional Medicine

Brenda Forsythe, DVM

Francesco Fortinguerra, Comitato Europeo Difesa Animali (CEDA)

Debbi Fouts, MS

Roger Fouts, PhD

Michael Fox, BVSc

Jane Ellen Geoff,PhD

Carol S. Ginandes, PhD

John Gluck, PhD, Emeritus Professor of Psychology and Psychiatry, University of New Mexico and Georgetown University

Caren R. Goodman, MD

Roberta Gray, MD

Jean Greek, DVM, DACVD, Board of Directors (AVAR)

Julia Gates Hartnell, MD, Radiologist

Susan J. Hall, DVM, Board of Directors (AVAR)

Lawrence A. Hansen, MD, Professor, Department of Neurosciences and Department of Pathology, University of California, San Diego School of Medicine

Bernard Herzog, Docteur en Médecine, Professeur d'Université (Médecine) en retraite, Association ARABEL

Professor Chris Hillier, Chair of Physiology, Glasgow Caledonian University

Barbara Hodges, DVM

Elliot M. Katz, DVM, President (IDA)

Paula Kislak, DVM, President (AVAR)

Jean-Jacques Kona-Boun, DVM, University of Montreal

Susan Kresbach, DVM

Dr. Rosanna La Carruba

Susan Lark, MD, Physician and Surgeon

Thierry Lesnick, Docteur en Biologie Moléculaire

Lorin Lindner, PhD, MPH

Dr. Marco Mamone Capria, University of Perugia

Clara Mancini, PhD, Research Fellow, Open University, UK

Diego Marchetti, PhD

Lori Marino, PhD, Emory University

Carmela Matera, MD, Medico

Bruno Mellano, Deputato Radicale della Rosa nel Pugno Membro XIII Commissione Agricoltura, Camera dei Deputati

Marta Meloni

Andre Menache, BVM, Scientific Advisor (Antidote Europe)

Dr. Laura Mencherini

Nancy Mroczek, PhD

Luca M. Palermo, MD

Nicole G. Paquette, Director of Legal and Government Affairs & General Counsel (API)

Annamaria Pascalicchio, Psychologist

Margaret Peppercorn, MD, Post Road Pediatrics, LLP

Mark Peppercorn, MD, Professor of Medicine, Harvard Medical School

Venessa Pero, MD

Alfred E. Peterson, MD, United Medical Associates

Renate Pilz, MD, Hematology-Oncology Physician

John J. Pippin, MD, Cardiologist, Senior Medical and Research Adviser (PCRM)

Theodore Polansky, DDS

Dr. Milos Polednicek, Thermodynamique Expert, RSI - IFP Group Company

Walter Rao, MD, DSS

Lara Rasmussen, DVM, DACVS, Former Director of Surgery Training, Western University of Health Sciences

Claude Reiss, PhD, Chairman (Antidote Europe)

Jeremy Rifkin, President (Foundation on Economic Trends)

Meredith Rives, DVM, Board of Directors (AVAR)

Professor Antonio Augusto Rizzoli

Tracie Romsland, DVM

Andrew Rowan, DPhil, Executive Vice President for Operations (HSUS)

Anne Ryleston, DVM, Canterbury Trails Veterinary Clinic

Chad B. Sandusky, PhD, Toxicologist, Director of Toxicology and Research (PCRM)

Helen Saraseca, BSc

Claudette Saulnier-Dyer, PhD, Neurochemistry

Melvin Schorin, MD, Cambridge Hospital Emergency Department

Jon Schumacher, PhD

Isabella Aurora Serini

Laurie Siperstein-Cook, DVM

Sheri Speede, DVM

Maria Sperada Laudisio, Pharmacist

Martin Stephens, PhD, Vice President for Animal Research Issues (HSUS)

Gordon Stull, VMD, Board of Directors (AVAR)

Deborah Tanzer, PhD, Psychologist

Paula Tatarunis, MD, Lawrence Memorial Hospital

Vicky Taylor, MD, MPH, Fred Hutchinson Cancer Research Center

Geza Teleki, PhD, Primatologist

Chiara Testi

Phyllis Troia, MD, Internal Medicine Physician

Nancy R. Tufford

Rich Ulmer, President and CEO, InVitro International

Robert Vergnani, MD, Ophthamologist

Margaret Walsh, PhD

Susanna Walsh, MD, FACOG, Boston University OB-GYN Associates

Neil C. Wolff, DVM, Founder and Treasurer(AVAR)

Paul Zveguinzoff, Psychologue clinician et psychothérapeute, Président d'Agir pour sa Santé