



**PETA**

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# **Citizens Petition to the Department of Health and Human Services to Adopt Modern Toxicity Testing Standards**

April 4, 2008

## **SUBMITTED TO**

The U.S. Department of Health and Human Services

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## **SUBMITTED BY**

People for the Ethical Treatment of Animals  
Jessica Sandler, Director, Regulatory Testing Division  
Susan L. Hall, Counsel, Regulatory Testing Division  
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AN INTERNATIONAL  
ORGANIZATION DEDICATED  
TO PROTECTING  
THE RIGHTS OF ALL ANIMALS



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Michael O. Leavitt, Secretary  
U.S. Department of Health and Human Services  
200 Independence Avenue, S.W.  
Washington, D.C. 20201

Re: Petition for Rulemaking to the Department of Health and Human Services  
to Adopt Modern Toxicity Testing Standards

Dear Mr. Leavitt:

On behalf of the more than 1.8 million members and supporters of People for the Ethical Treatment of Animals ("PETA"). We hereby petition the U.S. Department of Health and Human Services ("HHS") to initiate rulemaking.

## **I. STATUTORY AUTHORITY**

Pursuant to the Right to Petition Government Clause contained in the First Amendment of the Constitution, the Administrative Procedure Act ("APA"), 5 U.S.C. §553(e), and the Health and Human Services ("HHS") implementing regulations, the petitioners submit this citizens petition for rulemaking in the interests of protecting the public health and welfare, and furthering the objectives of the statutes cited below that pertain to animal testing.

## **II. ACTION REQUESTED**

The petitioners request that HHS and its institutes, the National Institutes of Health ("NIH"), the National Institute of Environmental Health Sciences, the National Center for Toxicological Research, and the National Institute for Occupational Safety and Health ("NIOSH"), commence rulemaking concerning animal testing by or for the National Toxicology Program ("NTP") that requires that the following criteria be satisfied:

1. There must be a showing that no alternatives to the proposed tests involving animals are available or will be available within the reasonably foreseeable future.<sup>1</sup>
2. There must be a showing that there is, or are, no existing studies, research or data on the subject of interest, or studies, research or data which are closely related to the subject of interest and can be extrapolated thereto (i.e. route-to-route extrapolation, QSAR Toolbox, *Medline*, *Scopus* and similar resources). Compounds and substances which are already known to be hazardous to

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<sup>1</sup> The reasonably foreseeable future should be no less than two years.

human health or to the environment are presumptively unsuitable for testing on animals.

3. There must be a showing to a reasonable degree of scientific certainty that the adverse effects caused to the animals are significantly outweighed by the expected benefits to be gained from the research.<sup>2</sup>
4. There must be a showing that there are no existing human data and that any proposed animal tests have relevance to human health effects, and that the results of such tests will be predictive of human outcomes.
5. There must be a sworn affidavit executed by the principal investigator certifying to compliance with the foregoing under penalty of perjury.

### III. BACKGROUND: NTP

The NTP was established in 1978 for the purposes of: 1) coordinating toxicology testing among federal agencies; 2) reinforcing the scientific underpinnings in toxicology; 3) developing, validating, and improving testing methods; and 4) providing data about toxic substances to health, research, medical and scientific stakeholders, as well as to the public. Three federal agencies provide the nucleus of the NTP, namely the National Center for Toxicological Research, the National Institute of Environmental Health Sciences and the National Institute for Occupational Safety and Health.

The NTP's mission statement appears on its web site and in a publication entitled *Current Directions and Evolving Strategies* (2006):

The NTP is an interagency program within the U.S. Department of Health and Human Services (DHHS) whose mission is to evaluate environmental substances of public health concern by developing and applying tools of modern toxicology and molecular biology. The program maintains an objective, science-based approach in dealing with critical issues in toxicology and is committed to using the best science available to prioritize, design, conduct, and interpret its studies. To that end, the NTP is continually evolving to remain at the cutting edge of scientific research, and to develop and apply new technologies.

Accordingly, the NTP's primary purpose is to characterize chemicals and other compounds that involve public health issues. One way in which the NTP discharges its mandate is through the solicitation and review of nominations of substances for toxicological study.

Any member of the public is eligible to nominate a chemical substance for NTP study. Even anonymous nominations are eligible for review.<sup>3</sup> The NTP reviews nominations and

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<sup>2</sup> *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986*, Section 5(4). 2000. Available at <http://www.archive.official-documents.co.uk/document/hoc/321/321-xa.htm>.

solicits and considers public comment on them. The Executive Committee of the NTP reviews the nominations, the public comments and any other relevant material and forms its own recommendation (i.e., test the substance, not test, or defer testing until further data is compiled and evaluated).

When a substance is ultimately selected for an NTP study, it generally entails testing for human health effects such as general toxicity, developmental and reproductive toxicity, genotoxicity, immunotoxicity, neurotoxicity, and carcinogenicity. None of the testing assays for those endpoints which rely on animal models has been scientifically validated according to internationally agreed upon standards as all *in vitro* tests must be.<sup>4</sup>

Many substances that have undergone NTP study are known toxicants, carcinogens, or substances whose hazardous properties are well understood and documented, or compounds for which animal models are entirely irrelevant. For example, the following substances, all of which have been robustly studied and researched, have been nominated for NTP studies: Prozac, butter flavoring, asbestos, and ethylene glycol.

#### **IV. SIGNIFICANT EVENTS AND STATUTORY CONSIDERATIONS**

The following brief chronology of significant events addressing alternatives to animal use illustrates the growing attention that has been given to non-animal methods in the U.S. and internationally during the past quarter century:

##### ***United States:***

- 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 (organized jointly by the U.S. and Germany and annually thereafter hosted by a different country each year).
- 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 recommends the Local Lymph Node assay as a reduction method for skin sensitization.
- 2000: The ICCVAM Authorization Act of 2000 (Public Law 106-545) creates ICCVAM as a permanent committee under the National Toxicology Program (NTP).

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<sup>3</sup> The response received to a Freedom of Information Act request dated June 16, 2004, indicated that fluoxetine (Prozac), was nominated for further study by a single, anonymous individual and “no reason for the nomination [was] provided.”

<sup>4</sup> See Section V.B. *infra* for Knight, A. Systematic Reviews of Animal Experiments Demonstrate Poor Human Clinical and Toxicological Utility. *ATLA* 35(6) (2007) 641-659.

- 2000: ICCVAM recommends the Up/Down procedure for reducing the number of animals used in acute toxicity tests.
- 2000: ICCVAM recommends 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: ICCVAM recommends the internationally validated non-animal methods EpiSkin™ and EpiDerm™ for skin corrosion.
- 2005: The NTP begins a formal collaboration with the NIH Chemical Genomics Center (NCGC) to develop high-throughput screening methods for toxicological testing.
- 2007: ICCVAM recommends the internationally validated method Bovine corneal opacity permeability (BCOP) test for eye corrosion.
- 2007: The National Academy of Science publishes a landmark report entitled "Toxicity Testing in the Twenty-first Century: A Vision and a Strategy"
- 2008: The EPA and NIH announce a Memorandum of Understanding to develop high-throughput non-animal methods for toxicity testing.

***International:***

- 1986: The European Union Council Directive 86/609/EEC mandates the use of and encourages development of non-animal methods.
- 1989: Creation of the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) funded by the German government to develop and promote regulatory acceptance of non-animal methods for toxicity testing.
- 1992: The European Centre for the Validation of Alternative Methods (ECVAM) is established as a unit of the Environment Institute, part of the Joint Research Centre (JRC) to research, validate and promote regulatory acceptance of non-animal methods for toxicity testing.
- 1993 and 1998: The European Union Cosmetics Directive 76/768/EEC 6<sup>th</sup> and 7<sup>th</sup> Amendments mandate a phase-out of animal testing for cosmetic ingredients and finished products by 2009.
- 1997: ECVAM validates an *in vitro* method of monoclonal antibody production.
- 1998: ECVAM validates the non- animal 3T3 neutral red uptake (NRU) phototoxicity test and the non-animal methods EpiSkin™ and EpiDerm™ for skin corrosion
- 1999: ECVAM accepts the Local Lymph Node assay as a reduction method for skin sensitization.
- 2000: ECVAM accepts Corrositex™ non-animal test for skin corrosivity and validates non-animal methods for measuring the potency of vaccines.
- 2002–2012: The European Commission Sixth Framework Program for Research and Technological Development (FP6) promotes and funds research into non-animal methods for specific areas of toxicity testing.
- 2002: ECVAM validates three *in vitro* methods for embryotoxicity.
- 2002: OECD approves Test Guidelines for 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.
- 2004: The U.K. parliament establishes the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) that provides funding and education for research that focuses on refinement, reduction, or replacement of animals in experiments.
- 2006: ECVAM validates methods for genotoxicity, neutropenia, a reduction method for acute toxicity in fish, and five *in vitro* methods for pyrogenicity.
- 2007: ECVAM validates methods for eye corrosion, skin irritation, and a reduction method for the LLNA skin sensitization test.

Two particularly significant events occurred in 2007 and 2008. In June 2007, the National Research Council of the National Academy of Sciences issued a report entitled "Toxicity Testing in the Twenty-first Century: A Vision and a Strategy" (the "Strategy Report"). The Strategy Report notes that animal-based toxicity testing is deeply entrenched in the research industry, but its relevance to humans is questionable. The Strategy Report underscores the need to take a progressive approach to testing the safety of chemicals, pesticides and other compounds, an approach that focuses on *in vitro* instead of animal studies. Such an approach should include studying human exposure data, using high throughput assays, focusing on toxicity pathways, developing models to describe responses in toxicity pathways and predict the human exposure necessary to produce responses in those pathways, and relying on human cell lines and cellular components instead of animals.

As the Strategy Report recognizes in its opening pages, "Change often involves a pivotal event that builds on previous history and opens the door to a new era." It continues:

Toxicity testing is approaching such a scientific pivot point. It is poised to take advantage of the revolutions in biology and biotechnology. Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin.

***This report ... envisions a major campaign in the scientific community to advance the science of toxicity testing and put it on a forward-looking footing.*** [Emphasis supplied.]

A second important event was the issuance of a Memorandum of Understanding – also known as an MOU -- between the NIH and the Environmental Protection Agency in February 2008. Pursuant to the MOU, high-speed, automated screening robots will be used to test suspected toxic compounds that may pose a risk to human health and the environment. These tests will utilize cells and isolated molecular targets instead of animal models. The collaboration between the EPA and the NIH is expected to increase the number of chemicals tested, reduce the costs and time required by animal based testing, and produce data that is far more relevant to humans. The MOU and the Strategy Report signal that the future of toxicity testing lies with *in vitro* methods and the demise of unreliable and irrelevant animal-based testing.

On the legislative side, Congress expresses the voice of the citizenry in the statutes it enacts. There are many laws that relate to the care, use, and welfare of, and testing on animals

ranging from the Animal Welfare Act, 7 U.S.C. §2131 *et seq.* of 1966<sup>5</sup>, to the National Institutes of Health Revitalization Act of 1993, 42 U.S.C. §283e, to the ICCVAM Authorization Act of 2000, 42 U.S.C. §2851 *et seq.*<sup>6</sup>

The Animal Welfare Act provides minimum standards for the care of animals used in laboratory research and experiments. However, the Animal Welfare Act is exceedingly limited in its protections since it specifically excludes mice, rats and birds, even though those species represent between 98-99% of the animals used in testing.

The NIH Revitalization Act directs the National Institutes of Health to conduct or support research into methods of research that “do not require the use of animals,” that “reduce the number of animals used in such research,” that encourage the “acceptance by the scientific community” of alternative methods, and that train “scientists in the use of such methods.” 42 U.S.C. §283e. It is clear from the language of the statute that Congress intended HHS to be an active contributor to the development and implementation of the above-mentioned plan since NIH is an operating institute within HHS. These provisions clearly demonstrate Congressional intent with respect to advancing the reduction, refinement, and ultimate replacement of animal testing.

The public policy considerations expressed in the ICCVAM Authorization Act, 42 U.S.C. §2851 *et seq.*, and one of the central objectives of that Act, are to promote and advance the reduction and replacement of animal testing, and to search diligently for alternatives. In establishing ICCVAM as a permanent Committee, Congress signaled its firm commitment to replacing live animal testing with *in vitro* methods. ICCVAM’s mandate is clear: reliance on animal-based methods must be reduced, refined and replaced.

Each of the referenced Acts expresses Congressional intent with respect to the reduction, refinement, and ultimate replacement of animal use in testing. Each Act positively supports the merits and spirit of this petition.

Despite the foregoing, the use of animals in toxicity, product safety and pharmaceutical testing has increased. For the year 2006, 1,012,713 animals were used in regulated animal testing -- a number which does not include mice, rats and birds, the animals most commonly used in toxicity testing. It has been estimated that for mice alone, some 100 million are used each year<sup>7</sup> and that approximately 15% of these animals are used in toxicity testing. These numbers reflect an enormous increase in the use of live animals in testing and research over the past two decades.<sup>8</sup> This trend stands in stark contrast to the laws cited above, to the future

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<sup>5</sup> The Animal Welfare Act has been amended four times in 1970, 1976, 1985 and 1990.

<sup>6</sup> See <http://www.hsus.org/web-files/PDF/legislation/CRS-07-animal-protection-fed-statutes.pdf> for a comprehensive listing of statutes relating to animals.

<sup>7</sup> Mukerjee, M. Speaking for the Animals. *Scientific American* August 2004

<sup>8</sup> The upward trend can only be expected to continue with the increase in production of genetically engineered animals and the huge national and international initiatives aimed at toxicity testing such as the High Production Volume Chemical Challenge and the Endocrine Disruptor Screening Program in the United States and the

outlook for toxicity testing embraced in the Strategy Report and the MOU, and to the ethical concerns that have brought animal testing to the forefront of public concern.

## V. ARGUMENT

### A. HHS and Its Institutes Have Not Discharged the Mandate of the NIH Revitalization Act of 1993 or the ICCVAM Authorization Act with Respect to Toxicity Testing Conducted by or for the NTP

#### 1. The Known Hazards of Artificial Butter Flavoring and Certain Components

Two of the most recent examples of the process gone astray are the nominations of artificial butter flavoring (diacetyl and acetoin) and asbestos fibers for NTP studies. Both substances are well-characterized, their risks are well understood, and further testing on animals will yield no scientifically valid information.

In July 2006, the United Food and Commercial Workers (UFCW) International Union nominated artificial butter flavoring and its ingredients, especially diacetyl and acetoin, to the NTP for toxicological characterization. At the June 22, 2007, NTP Board of Scientific Counselors Meeting, the nomination was approved, and a research concept was presented that included subchronic and chronic inhalation studies in mice.

When artificial butter flavoring is heated, the inhaled vapors can cause obliterative bronchiolitis (OB) – a rare, irreversible and life-threatening form of fixed obstructive lung disease. Since May 2000, the disease has been identified among workers in popcorn, flavoring and chemical manufacturing plants in several states. At least five of the affected workers have died, others are awaiting lung transplants and several have received damage awards against flavoring manufacturers for exposure-related injuries totaling more than \$50 million.<sup>9</sup>

Epidemiological studies of occupationally-exposed workers strongly implicate the volatile organic compound (VOC) diacetyl as the etiological agent in the OB cases reported. Diacetyl was the predominant compound found in the artificial butter flavoring and the indoor air of the popcorn manufacturing plant in which OB was first identified. Further, as cumulative exposure to diacetyl increased, the incidence of airway obstruction and abnormal results on spirometry also increased, demonstrating a clear dose-response relationship.<sup>10</sup> Importantly, reports of three bronchiolitis obliterans cases among former workers of a chemical plant manufacturing diacetyl narrow the disease's potential cause, since in contrast to the diverse

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Registration, Evaluation and Authorization of Chemicals in the European Union. See Knight, A. Systematic Reviews of Animal Experiments Demonstrate Poor Human Clinical and Toxicological Utility. *ATLA*. 2007; 35(6): 642.

<sup>9</sup> Brown, C. Popcorn Plant Workers Reach Settlement with Flavoring Maker Over Lung Problems. *Chemical Regulation Reporter*. 2005; 29(42): 1054.

<sup>10</sup> Kreiss, K. et al. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N Engl J Med*. 2002; 347(5): 330-338.



chemical exposures characterizing flavoring manufacture and use, exposure in the manufacture of diacetyl is limited to diacetyl and low concentrations of acetoin, acetic acid, and acetaldehyde.<sup>11</sup>

At the June 22, 2007, NTP Board of Scientific Counselors Meeting, the results of preliminary experiments in mice were reported. While inhalation of diacetyl caused deaths and acute necrotizing rhinitis, laryngitis and bronchitis, no lung or bronchiolar lesions were observed. Attempts to reduce nasal cavity toxicity – even including administration of diacetyl by oropharyngeal aspiration – all failed to produce bronchiolar lesions identical to OB. These results are consistent with earlier experiments in rats conducted by NIOSH scientists.<sup>12</sup> An extensive discussion of the anatomical and physiological differences between humans and rodents accounting for the observed results was also presented at the June 22 meeting by project leader Dr. Dan Morgan. For example, Dr. Morgan noted that rodent nasal turbinates are anatomically more complex and have a larger surface area relative to human turbinates, and it is for this reason that the rodent nasal cavity receives the highest inhaled dose of diacetyl and the greatest injury, while the bronchioles are protected. Dr. Morgan concluded that, in mice, most inhaled diacetyl reacts in the nasal cavity and bronchi and toxic concentrations do not reach the distal airways.

All of the panelists at the June 22 meeting expressed serious reservations regarding extrapolation of rodent data to humans. Dr. Katharine Hammond asked, “If only a very small fraction of an inhalation dose makes it to the bronchioles, how are we going to interpret that dose response? Certainly, we don’t want to take that large dose and say that’s the LOEL [Lowest Observed Effect Level].” Dr. Morgan agreed that “the most troubling issue is the use of the rodent in these studies.” Dr. Kenny Crump remarked “I think, realistically, that we have exposures already in the workplace, and we have results. I think it’s unlikely that the results here will overtake what we already know in humans, or will find out in humans, in actually setting exposure standards.”<sup>13</sup>

Despite the fundamental problem of extrapolation, despite the consensus that rodent models are inappropriate, and despite the acknowledgment that there are exposures in the workplace, the nomination of artificial butter flavoring and diacetyl for toxicological study was approved unanimously. Additional subchronic toxicity studies in mice by whole body inhalation exposure were proposed.

Several widely-reported events have recently re-focused public attention on the health hazards posed by artificial butter flavoring. Citing an EPA study of consumer exposure that has

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<sup>11</sup> Kreiss, K. Flavoring-related bronchiolitis obliterans. *Curr Opin Allergy Clin Immunol*. 2007; 7: 162-167.

<sup>12</sup> Hubbs, A. F., et al. Necrosis of Nasal and Airway Epithelium in Rats Inhaling Vapors of Artificial Butter Flavoring. *Toxicol Appl Pharmacol*. 2002; 185: 128-135.

<sup>13</sup> NTP Board of Scientific Counselors Meeting. June 22, 2007. Video and transcript available at <http://ntp.niehs.nih.gov/go/29657>. In a stunning non-sequitur, Dr. Hammond stated, “How are you going to interpret your results? Whatever results we get from the animal studies, how will they be interpreted if inhalation route of exposure was used and the outcomes would be different than the outcomes that would be expected in humans for exactly the same exposure?... However, that does not lessen my enthusiasm for the need for the study.”

only recently been published,<sup>14</sup> several popcorn manufacturers have removed diacetyl from their products or are in the process of doing so. These voluntary restrictions, accounting for more than 80% of the microwavable popcorn market, will greatly reduce diacetyl exposure. In addition the first case of OB in a microwavable popcorn consumer was reported in July 2007. Airborne levels of diacetyl during microwave popcorn preparation in the patient's home were reported to be similar to those measured in the microwave oven exhaust area in the quality assurance unit of the popcorn manufacturing plant in which OB was first identified.

Considering the overwhelming human-based evidence linking diacetyl with OB, coupled with industry's voluntarily discontinuing its use, it is clear that diacetyl exposure should be regulated and that further animal testing will either delay regulation, produce useless or anomalous data, and will not advance or protect human health.

In California, Assembly Bill 514 would ban diacetyl from workplaces in the State by 2010 and is currently moving through the legislature. In addition, California's OSHA draft regulatory standard would reduce employee exposure to diacetyl and other flavoring ingredients by mandating engineering controls, such as local exhaust ventilation and closed transfer of chemicals, as well as work practices such as covering containers and minimizing spills. It would also mandate comprehensive worker respiratory protection for organic vapors and particulates and require companies to conduct spirometry screening. Similar measures have already proven effective in limiting exposure in follow-up studies of workers at the popcorn manufacturing plant in which OB was first identified.

At the federal level, diacetyl is still designated "generally recognized as safe (GRAS)" by the FDA, and there are no specific OSHA standards regulating flavorings-related lung disease or requiring that diacetyl exposures be controlled. Several legislative efforts are currently underway that would require regulation of diacetyl. In May 2007, Representative Rosa L. DeLauro (D-CT), chair of the House of Representatives Appropriations subcommittee that funds the FDA, urged the agency "to consider revoking the generally safe designation for diacetyl and removing it from the market." Legislation introduced by Representative Lynn Woolsey (D-CA) in June 2007 that has recently passed the House of Representatives would require OSHA to issue an emergency standard for diacetyl. In addition, similar engineering and work practice controls to those described above are identified. Since diacetyl was the predominant VOC found in the indoor air of the microwave popcorn plants evaluated by NIOSH scientists, and since the lowest reported concentration of diacetyl in these plants was just 0.2 ppm, maintaining exposures below this very low level will also effectively control exposure to any other airborne contaminants that may be contributing to the observed adverse health effects.

As PETA suggested in the public comments submitted to NTP on May 10, 2007, issuance of a standard like the one described in Representative Woolsey's bill, along with revocation of diacetyl's GRAS designation, is the sort of regulatory action that is required immediately. Given the acknowledged difficulties in extrapolating rodent data to humans, there can be no justification for subjecting more animals to meaningless testing of this recognized hazard. A more reasonable approach would be to issue an alert, as suggested by David Michaels of the

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<sup>14</sup> Rosati, J. A., et al. Emissions from Cooking Microwave Popcorn. *Crit Rev Food Sci Nutr.* 2007; 47:8, 701-709.

Project on Scientific Knowledge and Public Policy, requesting information from lung disease specialists regarding possible OB cases among heavy consumers of popcorn.<sup>15</sup> This would generate more human-relevant epidemiological data at low exposure levels.

If the rule proposed in this petition were enacted by HHS, the toxicity testing which NTP proposes on diacetyl would not be undertaken because it would be clear that such testing is unnecessary, the compound has been studied, there is a significant human population which has been exposed to diacetyl, and animal testing has already proven to be non-predictive of human health effects.

## **2. The Known Hazards of Asbestos and Other Fibrous Amphibole Minerals**

Asbestos and other fibrous amphibole minerals contaminated vermiculite from a mine that operated near Libby, Montana from the early 1920s until 1990. In December 2006, the EPA nominated the Libby amphiboles to the NTP for toxicological study. Other related atypical asbestos and mineral fibers were also identified as a lower priority for study. The Agency for Toxic Substances and Disease Registry ("ATSDR") expressed a similar interest at the March 2006 NTP Executive Committee meeting. At the June 2007 NTP Board of Scientific Counselors meeting, the nomination was approved. The NTP's preliminary study recommendations include subchronic and chronic nose-only inhalation studies in rats.

Inhalation of asbestos fibers may lead to fibrotic lung disease (asbestosis), as well as cancers of the lung, pleura, and peritoneum. The fact that the evidence for the role of asbestos in human lung cancer comes from studies of the cause of death of occupationally-exposed workers and not from animal studies is undisputed. In its Toxicological Profile for Asbestos, ATSDR summarizes more than 40 epidemiological studies that provide reliable exposure-response information on the inhalation effects of asbestos in humans.<sup>16</sup>

In contrast, the ATSDR observes that animal studies provide at most supporting evidence for the fibrogenicity of asbestos and cautions that extrapolation of exposure-response relationships for asbestos-induced lung fibrosis in animals to humans is not recommended due to the anatomical and physiological differences between animals and humans influencing the rates of lung deposition and clearance of asbestos fibers. The NTP's research concept document for the nomination presents a discussion of these differences noting, for example, that since rats are obligatory nose-breathers, whereas humans breathe through the nose and mouth, only a fraction of human-respirable fibers are also rat-respirable. In the recently released draft document *Asbestos and Other Mineral Fibers: A Roadmap for Scientific Research*, the NIOSH explains that fibers that are capable of being deposited in the bronchoalveolar region of humans cannot even be evaluated in animal inhalation studies due to differences between rodents and humans in

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<sup>15</sup> Michaels, David. Popcorn Lung Coming to Your Kitchen? The FDA Doesn't Want to Know. *The Pump Handle*. Sept. 4, 2007. (Available at <http://thepumphandle.wordpress.com/2007/09/04/popcorn-lung-coming-to-your-kitchen-the-fda-doesnt-want-to-know>).

<sup>16</sup> Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Asbestos. U.S. Department of Health and Human Services. 2001.

fiber deposition characteristics. Finally, the shorter life-spans of animals used in laboratories mean that fibers persist longer in humans and as a result, the number of cells at risk, and the number of cell generations, is much higher in humans than in rats.

Several researchers conducting meta-analyses of human and animal exposure data have concluded that inhalation studies in rats are not sufficiently sensitive to detect risks to humans exposed to mineral fibers. In 1995, Rödelsperger and Weitowitz found that a significant cancer risk from asbestos exists for humans at a fiber concentration 300 times lower than that needed to produce the same risk in rats.<sup>17</sup> Muhle and Pott (2000) reached a similar conclusion demonstrating that inhalation studies in rats need fiber concentrations over 100 times higher to produce the same lung cancer risk observed in humans, and about 1000 times higher to produce the same mesothelioma risk.<sup>18</sup> They go on to note that if the current animal protocol for testing synthetic mineral fibers were to be applied to asbestos fibers, their very high carcinogenicity, known from epidemiologic studies, would be unlikely to be detected.

Similar concerns were expressed at the June 2007 NTP Board of Scientific Counselors meeting. Dr. Agnes Kane made the following observations:

The problem with rats is that very few of those animals develop malignant mesothelioma. In one series from the RCC studies..., malignant mesothelioma occurred in only 1 of 69 rats exposed to chrysotile asbestos fibers at 10 mg/m<sup>3</sup> over a lifetime. So, if we only get mesothelioma in 1 out of 69 rats in the study, how can we do relative potencies or ranking of other fiber types when it's such a rare endpoint?... However, in the RCC studies – a parallel study with hamsters – hamsters do not develop lung cancer in response to fibers, but they are very responsive to induction of mesotheliomas... The RCC studies had a multi-dose amosite study and the high dose study had to be terminated because the animals developed diarrhea, and they were treated with high doses of antibiotics which may or may not have altered the endpoints.<sup>19</sup>

The NTP's research concept document for the nomination defines "asbestos" as a commercial term describing several silicate minerals where crystalline growth produces long, thin, separable fibers and lists the six commercially important asbestos minerals recognized by regulatory authorities. The NTP and ATSDR note that asbestos – in any form – is an established human carcinogen. In its nomination letter, EPA acknowledges that there is a considerable data set available regarding the adverse health effects and biological activity of commercial grade asbestos materials but claims that there is some question as to the toxicity of the Libby amphiboles relative to commercial asbestos.

There is no reason to suspect that results of experiments on the naturally occurring fibers in Libby vermiculite will differ substantively from those obtained using commercial asbestos.

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<sup>17</sup> Rödelsperger, K. and Weitowitz, H. J. Airborne Fibre Concentrations and Lung Burden Compared to the Tumour Response in Rats and Humans Exposed to Asbestos. *Ann Occup Hyg.* 1995; 39(5): 715-725.

<sup>18</sup> Muhle, H. and Pott, F. Asbestos as Reference Material for Fibre-Induced Cancer. *Int Arch Occup Environ Health.* 2000;73(Suppl): S53-S59.

<sup>19</sup> NTP Board of Scientific Counselors Meeting. June 22, 2007.

Several epidemiological studies have documented the toxicity of the amphibole asbestos minerals present in the Libby, Montana mine, showing clear exposure-response relationships and calculating similar standardized mortality ratios for asbestosis and risks of lung cancer and mesothelioma to other groups exposed to amphibole asbestos.<sup>20</sup> In addition, the potential health effects of winchite and richterite, the two major amphibole contaminants of Libby vermiculite, have been investigated *in vitro*, and an amphibole with similar elemental composition and structure, fluoroedenite, has been linked with asbestos-related mortality in a human population.<sup>21</sup>

The main rationale for proposing new animal studies in the NTP nomination, as well as in the NIOSH *Roadmap* (see footnote 22), is that the heterogeneity of fibers in the workplace, in which a range of sizes and types of fibers are present, limits the ability of epidemiological studies to evaluate the influence of fiber size, chemical composition, and biopersistence on toxicity. Both NTP and NIOSH argue that animal inhalation studies are needed to investigate the toxicity of specific sizes and types of fibers.

However, at a May 4, 2007, public meeting held to discuss the NIOSH *Roadmap*,<sup>22</sup> Dr. Wayne Berman, co-author of EPA's 2003 *Asbestos Risk Assessment Protocol*, demonstrated that this rationale is based on a misconception. Even when a range of sizes and types of fibers are present, as in workplace epidemiological studies, the relative potency of each size and type of fiber can still be calculated exactly. No additional information is obtained from animal studies in which pure fiber samples are used, since fundamentally the same calculations are necessary.

In fact, more information is likely to be obtained from workplace epidemiological studies in which a range of sizes and types of fibers are present. Studies in which pure fiber samples are used are not designed to detect interactions between different sizes and types of fibers. In addition, if the pure fiber sample chosen for a particular animal study yields no interpretable results, the effort has been wasted, along with the animals' lives. In contrast, if a range of sizes and types of fibers are present as in the workplace, the categories dividing the range can be redefined to test different hypotheses. Dr. Berman concludes that new animal studies would not necessarily be more informative than better characterizing the human exposures in existing epidemiology studies directly. "After all," he said at the May 7, 2007 meeting, "we are interested in disease among humans."

Dr. Emanuel Rubin of Thomas Jefferson Medical College echoed these observations, noting that although the risk of developing lung cancer following asbestos exposure is increased many fold by smoking, "in experimental animals, it has not been possible at all to produce lung cancer by inhalation of tobacco smoke." He concluded that "[t]his shows the discrepancy between experimental data and epidemiologic data." Explaining the importance of the surface properties of asbestos fibers, Dr. Rubin observed that these properties cannot be determined

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<sup>20</sup> Bandli, B. R. and Gunter, M. E. A Review of Scientific Literature Examining the Mining History, Geology, Mineralogy, and Amphibole Asbestos Health Effects of the Rainy Creek Igneous Complex, Libby, Montana, USA. *Inhal Toxicol.* 2006; 18:949-962.

<sup>21</sup> Bandli, B. R. and Gunter, M. E. 2006.

<sup>22</sup> Public Meeting on *Asbestos and Other Mineral Fibers: A Roadmap for Scientific Research*. May 4, 2007. NIOSH. Transcript available at <http://www.cdc.gov/niosh/docket/NIOSHdocket0099.html>.

simply by viewing the fiber. “[T]hat's why the epidemiologic studies are so important, because there are genetic differences between animals and man, exposure times, routes of administration, et cetera, et cetera.” He urged that “no decisions be made on the role of any type of fiber until good epidemiologic studies have been done.”

Another more human-relevant approach was suggested by Dr. Richard Corley’s presentation at a recent International Science Forum on Computational Toxicology held at EPA’s Research Triangle Park laboratories outlining the progress of the Respiratory Program at the Pacific Northwest Laboratory in Richmond, CA. Numerous models of human organs, including lungs, were described. Three-dimensional models of the architecture and tissue mechanics of the respiratory systems of rats, mice, rabbits, monkeys and humans have been developed using MRI imaging technologies. Their results clearly demonstrate what Dr. Corley described as the “tremendous differences in architecture and thus responses of the respiratory systems of laboratory animals and humans to a variety of gases, vapors or airborne particles.”<sup>23</sup> These modeling techniques could be applied to predict deposition of airborne fibers and potential toxicity in the human lung.

According to NTP’s standard protocols, each chronic toxicology/carcinogenicity study can be expected to require between 1,000 and 1,400 rats and mice.<sup>24</sup> When nose-only inhalation exposure is studied, animals are squeezed into inhalation tubes. The exposures for these studies may begin at just three weeks of age ostensibly to address concern for possible children’s exposures.

The demonstrated toxicity of the Libby amphiboles to humans supports the inclusion of all amphibole asbestos minerals in current asbestos regulations and, since more human-relevant approaches for studying the toxicity of these minerals exist, conducting these animal tests would waste resources, needlessly kill thousands of animals, and delay regulatory action designed to protect the public.

If the rule proposed in this petition were enacted by HHS, the toxicity testing which NTP proposes on asbestos and other fibrous amphibole mineral fibers would not be undertaken because it would be clear that such testing is unnecessary, asbestos fibers have been studied and are a known carcinogen, there is a significant human population which has been exposed to asbestos fibers, and animal testing has already proven to be non-predictive of human health effects.

## **B. Studies Based on Animal Test Models Make neither Good Science, nor Good Public Health Policy.**

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<sup>23</sup> Corley, R. A. Towards the Virtual Human: Development of Three Dimensional Organ Models for Human Health Risk Assessment. International Science Forum on Computational Toxicology. 2007. U.S. EPA. Available at: [http://www.2007comptoxforum.com/abstracts/abstract\\_corley.htm](http://www.2007comptoxforum.com/abstracts/abstract_corley.htm).

<sup>24</sup> National Toxicology Program. NTP 2-Year Study Protocol. Available at: <http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=36305D16-F1F6-975E-79776DAD38EC101E>.

Examples of compounds that were fully tested on animals and proved harmful to humans are legion. A recent case is Vioxx, which was tested on gray spider monkeys and showed no ill health effects and was heart protective in six species. Other substances for which effects observed in animal tests failed to accurately reproduce effects in humans include tobacco,<sup>25</sup> arsenic,<sup>26</sup> mercury,<sup>27</sup> chromium,<sup>28</sup> benzene,<sup>29</sup> phthalates,<sup>30</sup> dioxin,<sup>31</sup> atrazine,<sup>32</sup> the monoclonal antibody TGN1412,<sup>33</sup> and many other compounds.<sup>34</sup>

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<sup>25</sup> C.R.E. Coggins, A Minireview of Chronic Animal Inhalation Studies with Mainstream Cigarette Smoke. *Inhal Toxicology* 991-1002 (2002). This work was performed to verify whether or not the inhalation response to cigarette smoke in animal species for assessing carcinogenic potential in humans reflects the strong epidemiological evidence in human smokers. Coggins documents that "inhalation response to cigarette smoke in animal species for assessing carcinogenic potential in humans" does not correlate with observational and epidemiological studies in humans. After reviewing smoke inhalation studies in mice, rats, hamsters, dogs and nonhuman primates, Dr. Coggins concluded that "[s]ignificant increases in the numbers of malignant tumors of the respiratory tract were not seen. . ." The author further concluded that "[f]uture work should clearly concentrate on genetic susceptibility in smokers. . . [since] [s]uch an approach would offer both academic challenges and opportunities for prevention."

<sup>26</sup> See, *Science on Trial: The Human Cost of Animal Experiments* by Dr. Robert Sharpe on the web at (<http://vivisection-absurd.org.uk/errors.html>):

For some seventy years, researchers were unable to connect arsenic with cancer due to being unable to verify the suspicion with animal experiments. Although arsenic had been connected with cancer as early as 1809, a report published in 1947 stated that the many animal experiments which had been conducted had only produced 'doubtful results'. O. Neubauer, *British Journal of Cancer*, 1947, vol. 1, pp.192- 251.

Experiments continued after this date and, even by 1969, researchers acknowledged that while it was believed there was a connection between cancer and arsenic, animal experiments had not offered any supporting evidence for this. A. M. Lee and J. F. Fraumeni Jr., *Journal of the National Cancer Institute*, 1969, vol. 42, pp.1045-1052.

In 1977, a report yet again stated that animal experiments had not produced supporting evidence of a link. F. W. Sunderman Jr., in *Advances in Modern Technology*, vol. 2, eds. R. A. Goyer and M. A. Mehlman (Wiley, 1977).

It was not until the end of the 1980s that researchers were finally able to produce cancer in animals - nearly 200 years after the link had first been suggested.

See also, Pershagen G., Lung cancer mortality among men living near an arsenic-emitting smelter. *Am J Epidemiol*. 1985 Oct;122(4):684-94.

<sup>27</sup> May, M. Breathtaking Research. *Environmental Health Perspectives*, 2000. Vol 108(4):168-169. See also, <http://www.stopanimaltests.com/u-epawasted.asp>.

<sup>28</sup> See, *Sacred Cows and Golden Geese: The Human Cost of Animal Experimentation*, by C. Ray Greek, M.D., and Jean Swingle Greek. "A study of dye-workers showed a high incidence of bladder cancer. Doves of dyed lab animals failed to prove the rule. Chromium was found to be carcinogenic in humans but not in animals."

<sup>29</sup> Since benzene was widely used, e.g., for the manufacture of detergents, pharmaceuticals, etc., there was concern as there appeared to be a link with cancer. Animal experimentation did not support this view, (L. B. Lave, *The American Statistician*, 1982, vol. 36, pp.260-261.) and some fourteen animal trials failed to show any connection between benzene and cancer. (D. M. De Marini, et al, in *Benchmarks: Alternative Methods in Toxicology*, ed. M. A. Mehlman (Princeton Scientific Publishing, 1989). Therefore workers were put at considerable risk in view of there being no 'evidence' of a link. It was not until the late 1980s, after over-dosing animals with benzene that cancer was induced. See, also DeLore P, Borgomono C. Acute leukemia following benzene poisoning. *Journal de Médecin de Lyon* 1928;9:227-36.

<sup>30</sup> The report of the Chronic Health Advisory Panel of the Consumer Product Safety Commission found that the chemical diisononyl phthalate (DNIP), is "clearly carcinogenic to the rodent" but that DNIP appears to induce liver

A recent article published in the journal *Alternatives to Laboratory Animals* exposes the myth that animal-based testing is predictive of human health effects.<sup>35</sup> Knight designed his study to test the validity of the assumption that animal based research and testing is predictive of human health effects. In order to test that hypothesis, Knight performed a comprehensive review of the *Scopus* biomedical bibliographic databases "for published systematic reviews of the human clinical or toxicological utility of animal experiments." In only two of 27 published studies, were animal models determined to be "either significantly useful in contributing to the development of clinical interventions, or were substantially consistent with clinical outcomes, ... one of which was contentious."

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cancer in rodents by a mechanism not readily induced in humans under real-world exposure conditions involving consumer products. Therefore, the rodent data were disregarded and the human risk has been deemed "negligible" by the panel. (Werner K. Advisory panel finds plastic products with phthalate pose little, no human risk. *Chemical Regulation Reporter* 2001 Jun 25:1020.) Taken from: People for the Ethical Treatment of Animals. *Wasted Money, Wasted Lives: A layperson's guide to the problems with rodent cancer studies and the National Toxicology Program*. 2006.

<sup>31</sup> Discordance noted in developmental toxicity classifications between rodents and non-human primates (p.12; Schardein JL. *Chemically Induced Birth Defects*, 3<sup>rd</sup> Ed. Rev, 1109 pp. New York: Marcel Dekker, 2000), and in cellular responses in humoral immunity between mice and rats. (p. 26; Smialowicz RJ, et al. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations: differences between mice and rats. *Toxicology and Applied Pharmacology* 124, 248-256. 1994).

<sup>32</sup> Despite epidemiological evidence of a link to cancer in humans and its ban in a number of European countries, atrazine remains a widely used pesticide in the U.S., with 75 million pounds used on crops annually. In fact, the EPA downgraded atrazine's cancer designation from "probable" to "not likely" because it deemed the animal test results largely "not relevant to humans." Clearly, the U.S. EPA's failure to follow the lead of European countries in banning atrazine is not for lack of data. (<http://www.stopanimaltests.com/pdfs/Atrazine.pdf>)

The mode of action is described by Cohen (Cohen, et al. *Evaluating the Human Relevance of Chemically Induced Animal Tumors*. 2004. *TOXICOLOGICAL SCIENCES* 78, 181-186.):

In Sprague-Dawley rats, atrazine affects the hypothalamus, leading to inhibition of the LH surge during the estrous cycle. This results in persistent secretion of estrogen and prolactin, ultimately leading to mammary tumors. These hormonal changes do not occur in F344 female rats or in CD-1 mice, a species that also is resistant to the mammary carcinogenic activity of atrazine. Even if the human hypothalamus were affected by atrazine in a manner similar to the Sprague-Dawley rat, a totally different result, mainly, a hypoestrogenic state, would be expected. Thus, it can be concluded that the MOA for atrazine-induced mammary tumors in Sprague-Dawley rats does not apply to humans, and, like d-limonene, there is neither cancer hazard nor risk to humans for this tumor.

<sup>33</sup> Anon. (2006). Statement re: TGN1412. See [http://www.tegenero.com/news/statement\\_re\\_tgn1412/index.php](http://www.tegenero.com/news/statement_re_tgn1412/index.php).

<sup>34</sup> Knight, 643 lists a group of non-steroidal anti-inflammatory drugs such as benoxaprofen (Oraflex), fenclofenac (Flenac), zomepirac (Zomax), bromfenac (Duract) and phenyl-butazone (Butazolidin) which passed animal tests. Similarly, the following antibiotics produced no adverse effects in animals but serious side-effect in humans: chloramphenicol, clindamycin, and temafloxacin. According to FDA statistics, 92% of drugs that pass animal tests fail in clinical trials in humans. See, Lester Crawford, FDA Commissioner, News from *The Scientist* 2004, 5(1):20040806-03; Published 6 August 2004 "More compounds failing Phase I."

<sup>35</sup> Knight, A. 641-659.



In the Conclusion to his survey, Knight asserts what follows:

In only two of 20 reviews in which clinical utility was examined, did the authors conclude that the animal models were either significantly useful in contributing to the development of clinical interventions, or were substantially consistent with clinical outcomes and one of these conclusions was contentious. Seven additional reviews also failed to clearly demonstrate utility in predicting human toxicological outcomes such as carcinogenicity and teratogenicity. Consequently, animal data can be generally assumed not to be substantially useful for these purposes.

...

Despite the fact that they have not passed, and indeed, could not pass, the formal scientific validation process required of non-animal models prior to regulatory acceptance, most animal models are incorrectly assumed to be predictive of human outcomes. The consistent application of formal validation studies to all test models is clearly warranted, regardless of their animal, non-animal, historical, contemporary or possible future status. Experimental model choices should be based on such critical scientific review, with appropriate consideration also given to animal welfare, ethical, legal, economic and other relevant factors.

Likely benefits would include greater selection of models truly predictive for human outcomes, increased safety of people exposed to chemicals that have passed toxicity tests, increased efficiency during the development of human pharmaceuticals and other therapeutic interventions, and decreased wastage of animal, personnel and financial resources.

In addition, the poor human clinical and toxicological utility of most animal models for which data exists, in conjunction with their generally substantial animal welfare and economic costs, justify a ban on the use of animal models lacking scientific data clearly establishing their human predictivity or utility.<sup>36</sup>

### **C. Standards in European Countries Show That the U.S. Is Behind the Times Both Ethically and Scientifically**

This section presents excerpts and summaries of some European laws which illustrate that the U.S. is behind other Western countries in both science and ethics.

#### **1. European Council Directive 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 alternative test methods validated and approved, and stronger legislation). This document codifies the principles of the 3Rs, namely the refinement, reduction and

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<sup>36</sup> Knight, 654.

replacement of animal testing, and confirms the principle that it is scientifically and morally insupportable to harm animals when valid alternatives may be used instead.<sup>37</sup>

While it is impossible to attribute European advances in non-animal methods exclusively to any one piece of legislation, it is clear that Directive 86/609/EEC has had significant positive effects. In general, the most significant of these is to mandate non-animal alternatives into the political agenda in all EU countries. Because European Union 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 the European Centre for the Validation of Alternative Methods ("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

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<sup>37</sup> The European Union Directive 86/609/EEC (the "Directive") illustrates the broad scope of protections accorded animals used in research. Unlike the Animal Welfare Act in the U.S., the Directive covers all species of animals, including those most commonly used: mice, rats, and birds. Additionally, an investigator must satisfy a more stringent standard before testing on animals can be justified or approved. The following is an excerpt from the Directive.

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.

- 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.

In addition to ECVAM, the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) was established by the German government in direct response to Directive 86/609/EEC. The goal of ZEBET is to bring about the replacement of legally prescribed animal experiments with alternative test methods, to minimize the number of animals used, and to alleviate the pain and suffering of animals used in experiments. ZEBET performs research, validation studies and maintains a publicly-accessible database of alternative methods.

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.<sup>38</sup> In addition to ECVAM, Europe has several national 3Rs centers, with domestic programs dedicated to advancing non-animal methods.

In its national legislation enacted in 1986, the UK specified that all scientific procedures on vertebrate animals should be licensed by the government in advance and that a balance of cost to animals versus potential benefit that may arise from any program should be made before a license could be issued.<sup>39</sup> The cost to animals is defined in the legislation as "adverse effects," encompassing pain, distress and harm, and the legal responsibility for the cost-benefit assessment lies not simply with officials but with the Secretary of State for the Home Department – the most senior government minister in the department responsible for animal experimentation.

Additional advances in non-animal methods in the EU are reflected 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

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<sup>38</sup> European Commission. ECVAM (European Centre for the Validation of Alternative Methods). *Statement on the Scientific Acceptability and Practical Availability of In Vitro Methods for the Production of Monoclonal Antibodies*, Appendix 2. 1998.

<sup>39</sup> *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986*, Section 5(4). 2000. Available at <http://www.archive.official-documents.co.uk/document/hoc/321/321-xa.htm>.

announced it would no longer approve LD50 testing protocols, and, in 2002, the OECD officially deleted the traditional LD50 protocol in exchange for several refinement protocols.

Since its adoption in 1986, Directive 86/609/EEC has also helped spawn additional European legislation, most notably amendments concerning animal testing to the European Union's Cosmetics Directive, which were first introduced in 1992. This legislation is discussed below.

## 2. European Ban on Animal Testing

In 1993 the European Union signaled its commitment to sustained progress regarding alternatives to animals by legislating a ban on marketing cosmetics containing ingredients tested on animals. The ban was originally to become effective on January 1, 1998. This far-reaching legislation prohibited marketing in Europe of products tested on animals or which contained ingredients tested on animals, even if those products were manufactured outside Europe. Industrial chemicals were exempted from the ban.<sup>40</sup>

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.<sup>41</sup> This new Directive introduced 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).

Today, as a result of Directive 86/609/EEC the concept of alternatives to animal use is not only accepted as standard practice by EU-based researchers and industry, but required by virtue of EU membership. It has become almost axiomatic that the use of available non-animal methods is preferable in the pursuit of best practices.

On February 13, 2008, in Brussels, the European Commissioner for the Environment, Stavros Dimas, addressed Members of the European Parliament on the issue of alternatives to animal testing. In his address Mr. Dimas stated emphatically that "[t]he Commission is working hard to reduce the need to resort to testing on animals as well as improve the situation for animals

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<sup>40</sup> Directive 93/35/EEC of 14 June 1993 amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal of the European Communities*, L 151, 32 (1993).

<sup>41</sup> Directive 2003/15/EC of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal of the European Union*, L66/26 (2003).

still used in experiments. The ultimate goal for the Commission is to replace animal testing with alternative methods."

## CONCLUSION

This petition furthers the interests of sound science, human health, animal welfare, and principles of significant ethical concern. The HHS can advance each of those interests by enacting rulemaking that requires that all NTP testing adhere to the following standards:

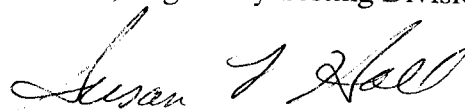
- There must be a showing that no alternatives to animal testing are available or will be available within the reasonably foreseeable future.
- There must be a showing that there is, or are, no existing studies, research or data on the subject of interest, or studies, research or data which are closely related to the subject of interest and can be extrapolated thereto. Compounds and substances which are already known to be hazardous to human health or to the environment are presumptively unsuitable for testing on animals.
- There must be a showing to a reasonable degree of scientific certainty that the adverse effects caused to the animals are significantly outweighed by the expected benefits to be gained from the research.
- There must be a showing that there are no existing human data, that any proposed animal tests have relevance to human health effects, and will be predictive of effects in human beings.
- There must be a sworn affidavit executed by the principal investigator certifying to compliance with the foregoing under penalty of perjury.

We urge the Agency to commence rulemaking in accordance with this petition.

Respectfully submitted,



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