



## Workshop Report\*

# Critical Evaluation of the Use of Dogs in Biomedical Research and Testing

Joanne Zurlo<sup>1</sup>, Kathryn A. Bayne<sup>2</sup>, Dorothy Cimino Brown<sup>3</sup>, Tanya H. Burkholder<sup>4</sup>, Vicki L. Dellarco<sup>5</sup>, Amy Ellis<sup>6</sup>, Laura Garrett<sup>7</sup>, Robert Hubrecht<sup>8</sup>, Erik R. Janus<sup>9</sup>, Lewis B. Kinter<sup>10</sup>, Elizabeth Luddy<sup>11</sup>, LaVonne D. Meunier<sup>12</sup>, Diana G. Scorpio<sup>13</sup>, James A. Serpell<sup>14</sup>, and Rory J. Todhunter<sup>15</sup>

<sup>1</sup>Center for Alternatives to Animal Testing, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; <sup>2</sup>AAALAC International, Frederick, MD, USA; <sup>3</sup>University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA; <sup>4</sup>Division of Veterinary Resources, Office of the Director, National Institutes of Health, Bethesda, MD, USA; <sup>5</sup>Office of Pesticides, US Environmental Protection Agency, Washington, DC, USA; <sup>6</sup>Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, USA; <sup>7</sup>University of Illinois, College of Veterinary Medicine, Urbana-Champaign, IL, USA; <sup>8</sup>Universities Federation for Animal Welfare, Wheathampstead, Hertfordshire, UK; <sup>9</sup>Stephoe & Johnson, LLP, Washington, DC, USA; <sup>10</sup>AstraZeneca Pharmaceuticals LP, Wilmington, DE, USA; <sup>11</sup>Center for Veterinary Medicine, US Food and Drug Administration, Rockville, MD, USA; <sup>12</sup>GlaxoSmithKline, King of Prussia, PA, USA; <sup>13</sup>Department of Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD, USA (current address: California National Primate Research Center, University of California, Davis); <sup>14</sup>University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA; <sup>15</sup>Cornell University College of Veterinary Medicine, Ithaca, NY, USA

In January 2011, the Johns Hopkins Center for Alternatives to Animals Testing (CAAT) hosted a workshop as part of its Transatlantic Think Tank for Toxicology (t4) on the use of dogs in biomedical research and testing. The Doerenkamp-Zbinden Foundation provided impetus for examining the use of dogs in particular because Mrs. Hildegard Doerenkamp (founder of the Doerenkamp-Zbinden Foundation) had a tremendous love and concern for dogs as human companions and desired to have CAAT initiate a program to reduce, refine, or replace their use in research and testing.

Dogs are beloved animals in many cultures. There is evidence of at least a 15,000-year association of dogs with humans, and dogs were the first species of animal to be domesticated. Humans and dogs have developed a symbiotic relationship over that time, with humans providing food and shelter to the dogs, and dogs providing aid in hunting, protection, and even warmth in early cultures of humans. In more recent years, especially after World War II, the number of dogs as pets has increased, particularly in North America, Europe, and Japan; in many situations, dogs are considered family members. According to the AVMA, more than 37% of US households have dogs as companion animals, numbering some 70 million. In contrast, in other human cultures and in populations greater than those mentioned above, dogs may be viewed as “unclean” and are not allowed to cohabitate with humans and/or are considered an agricultural commodity to be consumed as food.

Physicians and scientists also have used dogs as models to study various human and canine diseases. Early physiologists and physicians used dogs to study metabolic processes, because

dogs were available in the households and farms, are tractable, of a convenient size (compared to other agricultural species), and, based upon the written reports of public research demonstrations, there were no social mores against doing so. One consequence of these early researchers’ activities was the spawning of the anti-vivisectionist movement in the UK, whose members protested against the use of dogs for research purposes, especially since no anesthesia was used. In the last century and a half, research on dogs has resulted in major medical discoveries, such as the development of clinical ECGs, osmoregulation and body fluid homeostasis, and the discovery of insulin and its utility in managing clinical diabetes. Consequently dogs are still used to study human and canine diseases. In addition, dogs have been and continue to be used in safety testing of pharmaceutical, agricultural, and industrial chemicals, canine food products, medical devices, and other products. USDA data from 2007 indicate that just over 72,000 dogs were used in the US for all of these purposes. While most of these dogs are purpose-bred for research and not associated with the pet trade, it is nonetheless difficult for some people to reconcile their use in light of their historical and cultural association with dogs as companions.

CAAT brought together experts to closely examine the current use of dogs in both research and product safety testing, to determine the current rationale for their use in these procedures, to address the question of whether there are alternatives to their use, and to apply the principles of the 3Rs with the goal of replacing dogs if possible, dramatically curtailing their use where appropriate, and eliminating any pain or distress they might experience in areas where their use is indispensable.

\*a report of t4 – the transatlantic think tank for toxicology, a collaboration of the toxicologically oriented chairs in Baltimore, Konstanz and Utrecht sponsored by the Doerenkamp-Zbinden Foundation



### *The human-dog relationship*

Dr **James Serpell** opened the workshop with a presentation about why dogs are different, providing a history of the domestication of the dog from the wolf dating from 15,000-40,000 years ago. There are several hypotheses surrounding how and why dogs and humans developed a symbiotic relationship. Dogs may have begun to associate with humans as scavengers, or they may have cooperated while hunting. However, there are a number of reasons why these hypotheses are probably not correct. The most plausible hypothesis is that humans captured, tamed and kept young wolves as companions and social support providers. The domesticated dog then evolved by breeding and selection for those animals that were relatively tame and sociable. The selective forces for current dog breeds are morphology and behavior to fit into companionship or social roles and to fit into working roles. Social companionship with dogs has been shown to have both mental and physical health benefits for humans. Dog behaviors compatible with human social needs have positive effects on human attachment and well-being; bad behaviors tend to disrupt these attachments and are frequently reasons for owners to surrender a dog to a shelter. Studies of cognition in dogs have shown that dogs are adept at reading human social cues and that dogs are genetically predisposed to looking at humans. Since dogs are products of selection for anthropomorphic traits (Serpell, 2003), it is necessary to reevaluate the human social interaction with dogs in a laboratory environment. Optimal treatment of dogs in a laboratory environment is essential for continued public acceptance of the use of dogs for research purposes. In general, public approval for the use of dogs and nonhuman primates in North America and Europe has declined, most likely due to the increased status of dogs in Western society. Dr Serpell raised the question about the appropriateness of the dog as a research model since it has evolved to be hypersensitive to anthropogenic influences.

### *The use of dogs in biomedical research*

Dr **Tanya Burkholder** spoke next about some of the research areas in which dogs are used. She first pointed out some of the historical use of dogs for biomedical research, such as digestive physiology, operant conditioning, and cardiovascular studies. She (as well as other speakers) outlined why dogs have been so useful in research, namely because they were/are readily available, their large size makes certain types of research easier, they are easy to house and handle, and their anatomy and physiology are, in many ways, similar to that of humans.

Dogs continue to be used in research because they share many hereditary diseases with humans; there are 220 homologous hereditary diseases with uniform genetic mutations. Their outbred genetic background can mimic the human situation, and many therapies developed in dogs are translatable to humans. For example, dogs with hemophilias A and B, von Willebrand disease, and Factor VII deficiency are phenotypically similar to humans with the same diseases (Nichols et al., 2009). The dog is also a good model for leucocyte adhesion disease (LAD), a generally fatal disorder characterized by a defect in CD18 adhesion factor on neutrophils, rendering the neutrophils unable to migrate to a site of infection. Babies born with LAD succumb to bacterial infections and usually die by age twenty; dogs with LAD show

a similar clinical course and typically die by six months of age. Gene therapy represents one of the only viable treatments for this disease and has been successfully used in dogs with LAD (Bauer et al., 2009). Duchenne muscular dystrophy is a common disease in boys and results from loss of the protein dystrophin. A similar genetic defect occurs in the Golden Retriever breed. Hence these dogs are used to investigate stem cell and gene therapy interventions. Other human diseases for which there are canine counterparts are Alport syndrome, in which there are collagen defects in the glomerular basement membrane leading to kidney failure; retinitis pigmentosa, which is a group of hereditary disorders leading to blindness; and narcolepsy. Dogs are used in other research areas because of the similarities in the anatomy and physiology between dogs and humans. These areas include the study of Alzheimer's disease in aged Beagles; an induced asthma model in Beagles; and cardiomyopathy in Portuguese Water Dogs.

In summary, dogs are valuable models for biomedical research because they share many homologous diseases, and due to similarities in anatomy and physiology to humans, therapeutic interventions studied in dogs can be rapidly translated into human clinical trials.

### *The use of dogs in testing of pesticides*

The primary areas in which dogs are used for testing include safety evaluations for pesticides and drugs, as well as drug development. Dr **Vicki Dellarco** and **Erik Janus** gave presentations representing the EPA and industry perspectives on the use of dogs for regulatory purposes, respectively. The US Environmental Protection Agency (EPA) regulates the testing of pesticides and requires that pesticides be tested in one rodent and one non-rodent species. The dog is often used as the non-rodent species to assess systemic toxicity in subchronic and chronic studies and is also used for absorption, distribution, metabolism, and excretion (ADME) studies. Animal testing of pesticides is done to characterize the dose-response curve and to determine a reference dose (RfD), a dose below which the test substance is unlikely to produce adverse effects upon exposure. Retrospective analysis of pesticide data from the EPA showed that 40% of chronic RfDs are based on dog studies as compared to rodent studies. New regulations have been adopted in the US and Europe that eliminate the one-year chronic toxicity study for pesticides; the subchronic 90-day study is sufficient because a number of studies have shown that there is no additional significant information obtained from a longer study. Not all countries have adopted these new guidelines, and this has an impact on companies competing in global markets. If some countries still require a one-year study, then companies marketing to those countries will be required to provide these data, despite the requirements elsewhere. Thus, in order to reduce the number of dogs used for regulatory purposes on a global level, it will be necessary for all countries to harmonize their pesticide testing requirements.

### *The use of dogs in testing pharmaceuticals*

Dr **Lewis Kinter** from the pharmaceutical industry and Dr **Amy Ellis** from the US Food and Drug Administration (FDA) focused on the use of dogs for safety pharmacology and toxicity testing. Like the EPA, the FDA requires toxicity testing in one rodent and



one non-rodent species. The use of dogs in pharmaceutical product safety testing began in the wake of both the sulfanilamide elixir disaster that killed more than 100 children in 1937 and the enactment of the 1938 Federal Food, Drug, and Cosmetic Act, when dogs were already a prominent species in contemporary academic pharmaceutical research activities. Dogs continue to be used today as the non-rodent species in product safety bioassays, largely because of vast amounts of historical data in this species that aid in interpretation of the safety bioassay data and translation to outcomes in humans. Size, temperament, tractability, fecundity, and familiarity also contribute to the utility of dogs in pharmaceutical safety research. In particular, dogs are used for repeat dose toxicity studies and for safety pharmacology for QT prolongation. As with studies on pesticides, there is an ongoing debate on the need for chronic toxicity studies longer than three months; recently, the maximum duration of chronic studies for pharmaceuticals has been reduced from 12 to 9 months (ICH M3(R2), 2010). Regarding the usefulness of toxicity data from dogs relative to predicting human toxicity, both speakers referred to a study by Olson et al. (2000), a retrospective evaluation of data from 12 pharmaceutical companies. Data from compounds that showed human toxicity in Phase I, II, or III trials were reviewed with respect to their toxicity in animals. The study is just the first part of a two-part study, and it only looked at compounds that were either true positive or false negative in the animal studies. The study showed that only 71% of the human toxicities were detected in animal tests: 44% were detected in rodent tests (primarily rat) and 63% were detected in non-rodent tests (either dog or nonhuman primate). The combined data from rodent and non-rodent tests produced the 71% concordance.

Dr Kinter also outlined some of the new technologies under development for testing of pharmaceuticals. Among these are wireless physiologic data collection systems using telemetric devices. Numerous types of data can be collected using these devices, resulting in less pain and distress to the animals. Blocked rather than randomized study design has also resulted in a reduction in the number of animals needed. Therefore, while two species will still be required for FDA approval for the near future, newer and more sensitive techniques, e.g., imaging modalities, will allow for further reduction and refinement of animal use in this arena. In addition, careful study planning (e.g., thoughtfully considering study goals, discussing nonstandard protocols with regulators, using pilot data) can help limit the number of animals that are needed for testing.

#### *Research on dogs for the benefit of dogs*

To obtain a more complete picture of how and why dogs are used in research, several speakers were invited to present their research on dogs that both benefits dogs directly and that can be translated to human health. Dr **Dorothy Cimino Brown** described her research on assessing and treating chronic pain in dogs. She and her colleagues were successful in designing the Canine Brief Pain Inventory, which can be used to reliably quantify the dog owner's assessment of severity and impact of chronic pain in their animals. The instrument was based on questionnaires used for human patients, but is focused on dog owner observations of their pet's behavior. Dr Brown also presented the results of stud-

ies in which an activity monitor was used to assess behavior in normal dogs vs. dogs with bone cancer. From the tracings of the monitor, they are able to quantify the decreased daytime activity, as well as the impact of chronic pain on sleep patterns in these dogs with cancer. Another major part of Dr Brown's presentation focused on the use of a neurotoxin, resiniferotoxin, which is a relative of capsaicin, as a treatment for chronic pain in dogs with bone cancer. The drug was administered into the spinal fluid and, in essence, it targeted and then killed the chronic pain-sensing nerves to the leg with bone cancer. Two weeks post-injection, the animals' pain decreased markedly as assessed by a visual analog scoring system, and the effects were still evident after 14 weeks. The data from the dog studies was pivotal in the subsequent design of the clinical trial now open at NIH for human terminal cancer patients with intractable pain. This presentation demonstrated how research on pain alleviation in dogs was beneficial to the dogs themselves as well as to humans.

The second presentation was by Dr **Rory Todhunter**, who described the activities of the DNA bank at Cornell University. He and his colleagues are studying the genotypes and phenotypes of dogs whose DNA was provided by their owners. This information allows investigators to map inherited diseases; gene linkage studies have aided in identifying regions on chromosomes that contain genes responsible for certain diseases. To date, investigators have done more than 500 genome-wide screens on dogs and have found the mutations responsible for canine cone rod dystrophy. Some current studies include phenotyping of aged control dogs, mapping of the genes responsible for mast cell tumors in Labrador Retrievers, behavioral disease mapping, studies of inflammatory bowel disease in Boxers, and a multi-institutional oncology project looking at mast cell tumors, osteosarcomas, hemangiosarcomas, and T and B cell lymphomas. Phenotyping studies involve cardiac ultrasound, eye exams, orthopedic exams, body condition scoring, neurology exams, gait analysis, and mapping of tumor locations. By ruling out Labrador Retriever-specific inherited diseases, these dogs can become common controls for gene discovery for other investigators. Current methods to find genes underlying complex traits and diseases employ genome-wide association studies. Studies of genome-wide genotypes can substitute well for pedigree information and can be more accurate in establishing genetic relationships among individuals. Gene mapping studies are being done on several breeds to genotype hip dysplasia and osteoarthritis. From these studies, it may be seen that tremendous progress is being made in studying canine diseases, and since many of these diseases are similar to those in humans, the results will again translate across species lines.

Dr **Laura Garrett** gave the next presentation in this session, discussing the benefits of clinical trials in veterinary oncology. Tumors in companion animals account for 47% of death or euthanasia in dogs and 32% in cats. Given the prevalence of the disease, as well as better diagnostics, many cancers are curable with appropriate therapy. In addition, some cancers, e.g., lymphomas, are more like chronic diseases and can be managed. Many people, attached to their companion animals, are choosing to treat the cancers. Clinical trials for anticancer drugs are similar to those in humans. In Phase I, toxicity is assessed with increasing doses of the drug; in Phase II, the antitumor activity of the drug is evalu-



ated at maximally tolerated doses; in Phase III, the performance of the drug is compared to existing drugs. In addition, the dog studies serve as good pre-clinical studies to using these drugs in humans. As mentioned by other speakers, there are several dog models for human cancers – among these is canine appendicular osteosarcoma, in which dogs exhibit similar clinical behavior and genetics to children with pediatric osteosarcoma. There are 18 members of the Comparative Oncology Trials Consortium, and these centers are focused on companion animal malignancies as comparative models for human disease. The benefits of these studies are far-reaching. Companion animals and their caregivers benefit because new therapies are becoming available. People with comparable cancers for which dogs serve as a good model also benefit as these treatments are translated to human disease.

Finally, Dr **Elizabeth Luddy** outlined the requirements of the FDA for the approval of drugs intended for use in animals. A company intending to market a new animal drug must demonstrate the safety and effectiveness of the drug in the target species. Information on chemistry, manufacturing, and controls is also required to ensure that the drug will have and maintain the necessary quality, strength, purity, and identity. There is also a requirement that the labeling contain all necessary information for the veterinarian or animal owner to use the drug safely and effectively, including the risks associated with the drug. After approving a drug, the FDA continues to monitor the drug's safety and effectiveness, the manufacturing process, and the marketing of the drug to ensure that advertisements provide truthful information.

#### *The use of dogs in research and testing:*

##### *The AAALAC International perspective*

Dr **Kathryn Bayne** next gave a presentation on the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)'s perspective on the use of dogs in research and testing, highlighting the guidance given by AAALAC for the care and use of dogs in a laboratory setting. She emphasized the wording in the 7<sup>th</sup> edition of the *Guide for the Care and Use of Laboratory Animals (Guide)* (NRC, 1996) recommending that dogs be given opportunities for exercise by walking on a leash or having access to a larger area for social contact, play, or exploration. Social contact with other dogs and with humans is also beneficial to dogs in a laboratory environment. These conditions are considered by AAALAC in their evaluation of a laboratory animal program. She noted that the NRC report, *Laboratory Animal Management: Dogs* (1994), is also used as a Reference Resource by AAALAC in its assessment of programs with dogs.

The types of commendations given by AAALAC to institutions following on-site assessments of their animal care and use programs include the excellent health and condition of the animals, the high quality of the environmental enrichment program, the institutional culture of high quality animal care and welfare, the existence of a forum for staff on canine care and welfare, and detailed documentation of veterinary care. Dr Bayne also highlighted some of the findings from accreditation site visits related to canine care. Among these were the need for a periodic review of the canine exercise plan, a requirement to describe ongoing efforts to diagnose causes of diarrhea in the canine population at one institution, a rec-

ommendation for better temperature control for feed storage, and a recommendation to establish criteria for determining inadequate sanitation (such as colony forming units present on equipment after sanitation). Problems that have arisen during site visits outside the US (particularly in developing countries without national guidelines) relate to more basic issues such as single housing, insufficient opportunities for dogs to engage in normal species activities, undersized caging, inadequate veterinary care, inadequate sanitation control, and use of non-pharmaceutical grade drugs.

New standards presented in the updated *Guide* (NRC, 2011) address methods of restraint, for example, recommending systems that do not limit an animal's ability to make normal postural adjustments. Space recommendations call for enclosures that allow greater freedom of movement and less restricted vertical space. The need for exercise, positive human interaction, species-specific plans for housing and management, including environmental enrichment and social housing, are also reinforced. The new *Guide* also notes that certain species-specific behaviors can be used as indicators of pain or distress (e.g., vocalizations), but this requires that caregivers be trained to recognize clinical, behavioral, physiologic, and biochemical indicators of animal well-being. Current European guidelines (ETS 123), which are also used by AAALAC International in animal care and use program assessments, require that the design of indoor and outdoor enclosures allow for some privacy for dogs and allow them, at least in part, to control their social interactions. ETS 123 also recommends separate areas for different activities, which may be achieved by including raised platforms or pen subdivisions. These guidelines encourage and emphasize social housing and environmental enrichment. Therefore, through its global accreditation program and the application of these standards, AAALAC is in a unique position to encourage improved care and use standards for dogs in research and testing.

##### *Issues specific to working with dogs in research and testing*

This session focused on meeting the physical and behavioral needs of laboratory dogs. Dr **Robert Hubrecht** presented on the research techniques used to assess the well-being of dogs in a laboratory setting, including physiology, behavior, and cognitive state. He started by noting that housing should be based on a good understanding of the dog's natural history. Dogs are domesticated, social, macro-ostmatic (relying heavily on their sense of smell) omnivores, neophyllic (inquisitive), and, when feral, roam over large areas. Since dogs are domesticated and have been selected for their ability to interact with humans, they respond well to human social cues. Dogs should normally be housed socially. Study of their sensory systems shows that the dog's olfactory sense is from  $10^3$ - $10^8$  times more sensitive than that of humans, suggesting that olfactory enrichment might be useful. There is research indicating that giving dog-appeasing pheromone (DAP) can reduce fear. Other useful enrichment includes chews. Many toys/chews are brightly colored, but dogs are dichromatic and colors such as red are probably not very vivid to dogs. A significant welfare issue in some kennels is the loud noise from barking. Stress leading to distress induced by noise may cause physiological changes that are deleterious to the animals. However, appropriate kennel design in terms of the number of animals per room, pro-



viding visual sight lines (see below) and acoustic engineering of the rooms can produce much quieter rooms. Music is often used in kennels, but there is only limited research available regarding any benefits to the dogs. The fact that dogs may naturally range over large areas could explain why some dogs confined to small spaces in the laboratory setting exhibit behavioral abnormalities. These may include stereotypic behaviors, self-injurious behavior, or other signs of stress such as increased paw lifting, body shaking, yawning, or high cortisol/creatinine ratio. Social housing of the animals, human interaction, providing toys, and interacting with the dogs outside their home enclosure can mitigate some of this stress. It is well established that it is particularly important to provide puppies with a complex social and physical environment to allow a normal development. Another very important consideration is to design kennels so that the dogs have good visual access to both people and other dogs. At the same time, the design should provide the dogs with opportunities to control their social interactions. Visual barriers and other ways of providing complexity of the environment and choice to the dog should, therefore, be provided.

Drs **LaVonne Meunier** and **Diana Scorpio** presented on how dogs are cared for in research facilities in the pharmaceutical industry and in an academic setting. In the pharmaceutical setting, reproductively intact beagles are the most used species, and these are generally obtained from breeders. Breeders are assessed periodically for a number of quality assurance and animal welfare issues, including socialization and acclimatization programs. In either industry or the academic setting, animals are quarantined when first entering a facility. During that time, a veterinarian examines them and performs necessary diagnostics and behavioral assessments. Important preventive programs are typically in place, and these include regular health assessments, nail trimming, ear cleaning, and behavioral assessments. The dogs are vaccinated as required by state and local laws, weight is managed by feeding schedules and body condition scoring, and dental prophylaxis is performed as needed. Animals are kept in the environment that is best for their well-being and conforms to study requirements. As discussed by other speakers, social housing and human interactions are important, and the housing and environment allow for species-typical behaviors. Adequate staff training and behavioral management are important components of working with canines. Some companies allow dogs to be adopted when the dogs are no longer needed for study. While there are some challenges because dogs are not accustomed to living outside of a laboratory, this policy can be beneficial to both the dogs and their prospective owners.

#### *Applying the 3Rs to research and testing with dogs*

The panel discussion at the end of the meeting focused on how to reduce, refine, or replace dog use in research and testing. Clearly, some testing and research is done in dogs for historical reasons rather than because they are the best models. A number of participants pointed out that the choice of a model should be based on science, not on historical data. Most of the discussion revolved around the testing issue and the fact that toxicology lags far behind pharmacology in applying state-of-the-art science to the development of mechanistically-based models. It was also pointed

out that it is difficult to truly evaluate the usefulness of toxicity data obtained from dogs because the proprietary data are not readily available. Only recently have pharmaceutical companies volunteered to share their pre-clinical data for comparison with clinical trial data. Related suggestions were to develop a standard for non-clinical data and to require that data be available, even for a limited period of time. It is also essential to continually evaluate the usefulness of data obtained from dog studies, i.e., whether these data are being used to make regulatory decisions.

Research to replace dogs in testing is under way, but this is still at an early stage. The goal of replacing dogs is primarily financially driven, since dog studies are so expensive to conduct. Reduction and refinement of dog use are occurring with the use of more sensitive tests, telemetric methods, and improvements in ability to recognize and alleviate pain and distress. However, the best hope for the dramatic reduction and ultimate replacement of dogs in testing is in the implementation of the recommendations in the NRC report *Toxicity Testing in the 21<sup>st</sup> Century – A Vision and a Strategy* (NRC, 2007) where true advances in mechanistic toxicology using human cell systems will provide the basis for predicting human toxicity.

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Joanne Zurlo, PhD  
The Johns Hopkins Center for  
Alternatives to Animal Testing  
615 North Wolfe Street  
W7032, Baltimore  
MD 21205, USA  
e-mail: jzurlo@jhsph.edu