Animal experimentation under scrutiny: new studies yield disturbing results

Andrew Knight BSc., BVMS, CertAW, MRCVS, Animal Consultants International, www.AnimalConsultants.org, Feb. 2006

Animal Consultants International scientists have been critically examining various aspects of animal experimentation since 2004. Studies such as the following are presented at international scientific conferences and published in scientific and medical journals. Additional and future studies may be found at www.AnimalConsultants.org 'portfolio.' To arrange a presentation or project email info@animalconsultants.org.

Laboratory animal suffering

Balcombe J, Barnard N, Sandusky C. Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science* Nov. 2004;43(6):42-51.
Eighty published studies were reviewed to document the potential stress associated with three routine laboratory procedures commonly performed on animals: handling, blood collection, and gavage. Handling was defined as any non-invasive manipulation that is part of routine husbandry, such as picking up an animal, and/or cleaning or moving an animal's cage. Significant changes in stress indicators (e.g., concentrations of corticosterone, glucose, growth hormone or prolactin, heart rate, blood pressure, and/or behavior) were associated with all three procedures in the reviewed studies (reporting primarily on rats, mice, monkeys, dogs, rabbits, hamsters, bats, or birds). Studies showed that animals responded with rapid, pronounced, and statistically significant elevations in stress-related responses to each of the procedures examined. Changes from baseline or control measures typically ranged from 20 to 100 percent or more and lasted from 30 to 60 min or more. These findings indicate that laboratory routines are associated with stress, and that animals do not readily habituate to them. The data suggest that significant fear, stress, and possibly distress are predictable consequences of routine laboratory procedures, and that these phenomena have substantial scientific and humane implications for the use of animals in laboratory research.

Balcombe J, Barnard N. Laboratory environments and rodents' behavioural needs: a review. *Laboratory Animals* 2006. In press.
Laboratory housing conditions have significant physiological and psychological effects on rodents, raising both scientific and humane concerns. Published studies of rats, mice and other rodents were reviewed to document behavioural and psychological problems attributable to predominant laboratory housing conditions. Studies indicate that rats and mice value opportunities to take cover, build nests, explore, gain social contact, and exercise some control over their social milieu, and that the inability to satisfy these needs is physically and psychologically detrimental, leading to impaired brain development and behavioural anomalies (e.g., stereotypies). To the extent that space is a means to gain access to such resources, spatial confinement likely exacerbates these deficits. Adding environmental "enrichments" to small cages reduces but does not eliminate these problems, and we argue that substantial changes in housing and husbandry conditions would be needed to further reduce them.

Poor human predictivity

Bailey J, Knight A, Balcombe J. The future of teratology research is *in vitro*. *Biogenic Amines* 2005;19(2): 97–145.
Birth defects induced by maternal exposure to exogenous agents during pregnancy are preventable, if the agents themselves can be identified and avoided. Billions of dollars and man hours have been dedicated to animal-based discovery and characterisation methods over decades. We show here, via a comprehensive systematic review and analysis of this data, that these methods constitute questionable science and pose a hazard to humans. Mean positive and negative predictivities barely exceed 50%; discordance among the species used is substantial; reliable extrapolation from animal data to humans is impossible, and virtually all known human teratogens have so far been identified in spite of, rather than because of, animal-based methods. Despite strict validation criteria that animal-based teratology studies would fail to meet, three *in vitro* alternatives have done so. The embryonic stem-cell test (EST) is the best of these. We argue that the poor performance of animal based teratology alone warrants its cessation; it ought to be replaced by the easier, cheaper and more repeatable EST, and resources made available to improve this and other tests even further.

Knight A, Bailey J, Balcombe J. Animal carcinogenicity studies: 1. poor human predictivity. *Alternatives to Laboratory Animals* 2006;34. In press.
The regulation of human exposures to potentially carcinogenic chemicals constitutes society's most important use of animal carcinogenicity data. Environmental contaminants of greatest U.S. concern are listed in the Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) chemicals database. However, of the 160 IRIS chemicals lacking even limited human exposure data but possessing animal data as of January 1, 2004, we found that in most cases (58.1%; 93/160) the EPA considered animal carcinogenicity data inadequate to support a classification of probable human carcinogen or non-carcinogen. For the 128 chemicals with human or animal data also assessed by the World Health Organization's International Agency for Research on Cancer (IARC), human carcinogenicity classifications were compatible with EPA classifications only for those 17 having at least limited human data ($p = 0.5896$). For those 111 primarily reliant on animal data, the EPA was much likelier than the IARC to assign carcinogenicity classifications indicative of greater human risk ($p < 0.0001$). The IARC is a leading international authority on carcinogenicity assessments, and its significantly different human carcinogenicity classifications of identical chemicals indicate that: (i) in the absence of significant human data the EPA is over-reliant on animal carcinogenicity data, (ii) as a result, the EPA tends to over-predict carcinogenic risk, and (iii) the true predictivity for human carcinogenicity of animal data is even poorer than indicated by EPA figures alone. EPA policy erroneously assuming that tumours in animals are indicative of human carcinogenicity is implicated as a primary cause.

Knight A, Bailey J, Balcombe J. Animal carcinogenicity studies: 2. obstacles to extrapolation of data to humans. *Alternatives to Laboratory Animals* 2006;34. In press.

Due to limited human exposure data, risk classification and the consequent regulation of exposures to potential carcinogens has conventionally relied mainly upon animal tests. However, several investigations have revealed animal carcinogenicity data to be lacking in human predictivity. To investigate the reasons, we surveyed the 160 chemicals possessing animal but not human exposure data within the U.S. Environmental Protection Agency chemicals database that had received human carcinogenicity assessments by January 1, 2004. We found a wide variety of species used, with rodents predominating; a wide variety of routes of administration used, and a particularly wide variety of organ systems affected. The likely causes of the poor human predictivity of rodent carcinogenicity bioassays include (i) the profound discordance of bioassay results between rodent species, strains and genders, and further, between rodents and human beings; (ii) the variable yet substantial stresses caused by handling and restraint, and the stressful routes of administration common to carcinogenicity bioassays, and their effects on hormonal regulation, immune status and carcinogenesis predisposition; (iii) differences in rates of absorption and transport mechanisms between test routes of administration and other important human routes of exposure; (iv) the considerable variability of organ systems in response to carcinogenic insults, between and within species; and (v) the predisposition of chronic high dose bioassays towards false positive results, due to the overwhelming of physiological defences, and the unnatural elevation of cell division rates during *ad libitum* feeding studies. Such factors render attempts to accurately extrapolate human carcinogenic hazards from animal data profoundly difficult.

Alternatives to laboratory animal use

Knight A, Bailey J, Balcombe J. Animal carcinogenicity studies: 3. alternatives to the bioassay. *Alternatives to Laboratory Animals* 2006;34. In press.

Conventional animal carcinogenicity tests take around three years to design, conduct and interpret. Consequently, only a tiny fraction of the thousands of industrial chemicals in use have so far been tested for carcinogenicity. Despite the cost of hundreds of millions of dollars, millions of skilled personnel hours, and millions of animal lives, several investigations have revealed animal carcinogenicity data to lacking in human specificity (ability to identify human *non*-carcinogens), which severely limits its human predictivity. Causes include the scientific inadequacies of many carcinogenicity bioassays, and numerous serious biological obstacles, which render attempts to accurately extrapolate human carcinogenic hazards from animal data profoundly difficult. Proposed modifications to conventional bioassays have included the elimination of mice as a second species, the use of genetically-altered or neonatal mice, decreased study durations, initiation-promotion models, greater incorporation of toxicokinetic and toxicodynamic assessments, structure-activity relationship (computerised) systems, *in vitro* assays, cDNA microarrays for detecting genetic expression changes, limited human clinical trials, and epidemiological research. Potential advantages of non-animal assays when compared to bioassays include superior human specificity results, substantially reduced timeframes, and greatly reduced demands on financial, personnel and animal resources. Inexplicably, however, regulatory agencies have been frustratingly slow to adopt alternative protocols. In order to decrease cancer losses to society, a substantial redirection of resources away from excessively slow and resource-intensive rodent bioassays, into the further development and implementation of non-animal assays, is both strongly justified and urgently required.

Knight A. Humane teaching methods demonstrate efficacy in veterinary education. Under review.

Animal use resulting in harm or death has historically played an integral role in veterinary education, in disciplines such as surgery, physiology, biochemistry, anatomy, pharmacology, and parasitology. However, the last decade has seen a rapid increase in the availability of non-harmful alternatives, such as computer simulations, high quality videos, 'ethically-sourced cadavers' such as those from animals euthanased for medical reasons, preserved specimens, models and surgical simulators, non-invasive self-experimentation and supervised clinical experiences. However, experience has shown that many veterinary faculty remain opposed to such teaching methods, usually citing teaching efficacy as their main concern. Consequently studies were reviewed comparing learning outcomes generated by non-harmful teaching methods with those achieved by harmful animal use. Of ten studies from 1989 to 2000, nine assessed surgical training—historically the discipline involving greatest harmful animal use. 30% (3/10) demonstrated superior learning outcomes using more humane alternatives. 60% (6/10) demonstrated equivalent learning outcomes, and only one study demonstrated inferior learning outcomes. Eleven additional studies in which comparison with harmful animal use did not occur illustrated other benefits of humane teaching methods, namely; time and cost savings, increased repeatability and flexibility of use, customization of the laboratory experience, more active learning, facilitation of autonomous and life-long learning, improved attitudes towards computers and alternatives to animal use, and increased employer perception of computer literacy. The results indicate that veterinary educators can best serve their students and animals, while minimizing financial and time burdens upon their faculties, by introducing well-designed teaching methods not reliant upon harmful animal use.