Perspective

The Muddle of Models: What You Don't Know Can Hurt You

Donald G. Robertson*

Applied and Investigative Metabonomics, Bristol-Myers Squibb Company, Princeton, New Jersey 08543

Received June 5, 2008

Prior to clinical trials, animal models remain the primary tools by which the pharmaceutical industry demonstrates the efficacy and safety of candidate therapeutic agents. Despite this reliance, much remains unknown about the models that we use and the factors that affect how they perform. This ignorance can lead to inappropriate assumptions, questionable procedures, and incorrect conclusions from data generated in these models. This perspective provides a narrative of several such instances and provides recommendations as to how we might address our knowledge gap by better characterization of our models, better understanding of our practices, and better control of our studies.

Contents

1. Introduction

Over the past year, I have spent considerable time contemplating the models that pharmaceutical toxicologists and pharmacologists use in our work. As I was unemployed, or more correctly "between jobs", for a good deal of that time, one might cynically suggest that I had nothing better to do, but that was actually not the case. Several projects I have been involved with over the past several years, both with my previous employer and now with my current employer, have lead me to question some very basic assumptions about the animal models that we use in our work. While my experience is in pharmaceutical development, I imagine that my observations are not unique to that industry, although the examples I cite will be.

While in vitro technology has made tremendous advances over the past decade, whole animal in vivo studies still remain the "bread and butter" of our industry. There are many reasons for this, but debating the comparative virtues of in vivo vs in vitro work is not the intention of this perspective. Rather, the inherent vagary of conducting in vivo work is. One can debate about how successful (*1, 2*) or not (*3, 4*) we are as an industry at predicting potential human toxicity using our preclinical animal models. However, regardless of where one stands on that issue, it can always be argued that we can do better. While the consequences of taking a false negative forward into clinical development is by far our greatest concern, the loss of therapeutic (and economic) potential due to inappropriate termination of a compound also has significant ramifications. Animal models still remain the primary means by which we predict human efficacy and safety prior to actual clinical trials. Better models mean better predictions, which will lead to better attrition statistics (*5*) and lower probability of postmarket withdrawal.

One of the main reasons my concern level about models has been heightened in recent years is due to the metabonomic technology that I now utilize routinely in my work. Metabonomics (also known as metabolomics or metabolic profiling) involves the comprehensive assessment of small molecules (not including proteins and nucleic acids) in biological samples (*6*). Like other "omic" technologies (e.g., transcriptomics and proteomics), metabonomics has a shocking disregard for the results you want to see, but forces you to look at what actually happens, which probably includes all kinds of findings that you might wish you had never generated.

1.1. Example 1: The Impact of Study Design. A somewhat humorous (and unpublished) example of this involves some early work that we were doing to try and identify biomarkers of osteoarthritis (OA). We were working with our pharmacology colleagues who were using a matrix metalloproteinase 13 (MMP13)-injected knee rat model of OA (*7*). The study was not run with concurrent control animals, but rather, pretest samples were used for comparison for our urinary metabonomic analyses. After running a cohort of urine samples, my analytical colleague called me, indicating that he had identified a novel (at least to us) nuclear magnetic resonance (NMR) peak that seemed to consistently disappear with time after initiation of cartilage degradation by the MMP13 injection. Could this be the elusive OA biomarker, disappearing with the progression

* To whom correspondence should be addressed. Tel: 609-252-5661. E-mail: don.robertson@bms.com.

10.1021/tx800204g CCC: $40.75
Published on Web 08/26/2008

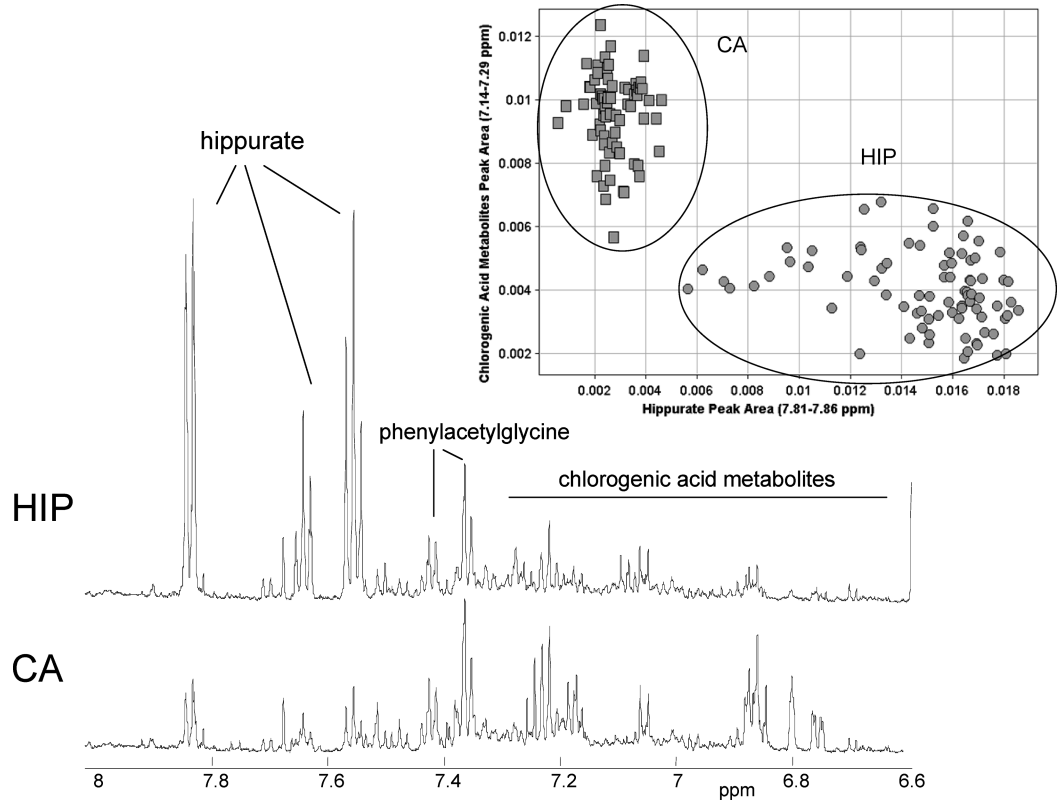

Figure 1. NMR spectrum (600 MHz) of rat urine consistent with historically obtained spectra (upper trace, HIP). The spectrum is characterized by relatively high levels of hippurate with lower levels of chlorogenic acid metabolites. The lower tracing is an aberrant spectrum obtained from atypical rat phenotype (CA). The spectrum has much lower levels of hippurate and higher levels of chlorogenic acid metabolites than seen in typical rat spectrum. Inset: Plot of peak area integration of hippurate vs chlorogenic acid metabolites, demonstrating complete resolution of the phenotype. Each filled circle represents an individual sample data point from either a HIP- or CA-phenotype rat (phenotype clusters are circled). Adapted from *12*; used with permission.

of the disease? After some diligent identification work, my colleague called me indicating that he had identified the peak as phloretic acid. While this compound might be quite familiar to agricultural or nutritional biochemists, we were unfamiliar with it and had to look it up. Imagine our surprise when we found that it was a gut flora-derived metabolite of phloretin or its glucoside phloridzin (*8*), major constituents of apple peels (among other things) (*9*). An apple peel metabolite as an inverse biomarker of OA? We decided not to pack our bags for Stockholm. As it turned out, our colleagues in pharmacology had not sufficiently acclimated their animals before putting them on study. What we were measuring was the disappearance of a diet-derived metabolite, as apple products were evidently in the diet supplied by the animal vendor but not in our in-house diet. While this makes for an amusing anecdote and makes a good case for proper animal acclimation or the use of concurrent controls, the "rest of the story" is that phloridzin has been extensively studied for its glucose-regulating activities (*10, 11*). Had this been a diabetes program instead of OA, the ramifications may have been a bit more significant.

1.2. Example 2: Are We Getting What We Think? An example of muddled models with wider relevance involves work where we identified an unusual phenotype (as determined by urinary metabolic profile) in Sprague−Dawley rats coming from a specific room (designated as a colony) at a facility of a well-respected animal supplier (*12*). Metabolically, the phenotype of the animals could be completely resolved by measurement of urinary hippuric acid- and chlorogenic acid-related metabolites alone (Figure 1). We were not looking for this; it was just an anecdotal finding in several of the studies that we were conducting at the time. We monitored this phenotypic difference for over a year, during which time the phenotype was stable, although it eventually disappeared (*13*). We suspected that the difference was due to unusual gut microflora and later confirmed that the origin of the phenotypic difference was most likely due to husbandry practices where rat colonies, which were originated with limited gut flora, were prevented from acquiring normal gut bacterial speciation due to meticulous hygiene practices (*14*). It should be emphasized that the magnitudes of the metabolic changes between the phenotypes were not trivial, exceeding an order of magnitude in some cases. My speculation is that this type of model variance has probably happened many times in the past, regardless of the animal vendor, and is probably not an unusual occurrence across the industry. What has changed is that we now have tools where these otherwise subtle differences are readily detected.

What I remember most about this incident were the reactions I received from several groups I spoke to about this finding. Generally, they went something like "What the @#$&! do you expect us to do about this?". Indeed, it is an important question—what do we do? It could be argued that we have stumbled along for years in blissful ignorance of this type of finding—why worry about it now? If I never had run a study that failed to reproduce previous findings or never had that single low-dose animal that compromised a safety margin, I might tend to agree. However, that has not been my experience. Perhaps a bit more scrutiny of the models that we use and how we use them is warranted. I would argue, even when we choose to ignore the findings, conscious disregard is always superior to blissful ignorance.

2. Underlying Issues

When contemplating the issue of muddled models, several lines of reasoning must be considered. How well-characterized are our models, how well thought out and understood are our practices, and how well-controlled are our studies?

2.1. Model Characterization. Within the pharmaceutical industry, you may get very different answers to this question from pharmacology groups as compared to regulatory toxicology groups. Pharmacology groups tend to use models that vary from program to program and evolve as the programs or the science behind the programs evolves. This is quite appropriate but may lead to a lot of "apples and oranges" type comparisons of study results generated within a program at different times or between different programs and particularly between pharmacology and toxicology groups.

Within regulatory toxicology groups, the opposite is almost always the case as we become committed to relatively few models because of the need for a robust historical archive of clinical, clinical pathology, and histopathology data that is necessary for interpreting safety data. Problems can occur when we try to "multipurpose" studies in an effort to reduce animal usage and speed up development timelines. It is attractive to obtain toxicology end points while we are conducting in vivo pharmacology experiments. Additionally, the case has been and still is being made that in many instances toxicologists would be better off conducting their safety studies in pharmacological models or models of human disease (*15, 16*), the hypothesis being that these models might better predict human response as they better capture the pharmacodynamic, pharmacokinetic, and physiological status of the human target population. While this may be true in some instances, routine use of such models in the toxicological setting is best served if the model has been robustly characterized. The reasoning of traditional toxicologists in utilizing standard models is still valid. The better characterized a model is, the more readily can departures from "normality" be detected. For example, is one instance of bile duct hyperplasia in a mid dose drug treatment group an example of a toxic effect or is it a feature of the model (i.e., a background lesion)? Resolving this type of question is a common (and often critical) occurrence in our profession and is only addressable (short of running additional studies) if we have well-characterized models. The question of model characterization can be further subdivided into two parts: those things that we know about the model (but may choose to ignore) and those things that we do yet not know.

2.1.1. Example 3: Impact of Diet. An example of conscious model manipulation that caused us some consternation involves some investigative work that we were doing into statin-induced myopathy. We were conducting studies evaluating the effect of creatine modulation on cerivastatin-induced myotoxicity that involved administration of copious amounts of creatine or the creatine analogue β-guanadino propionic acid (βGPA)—so much so that we administered them as admixtures in the diet (*17*). As the diet we were using at the time (Purina 5002) was not a defined diet, we switched to the AIN93G diet (*18*), a purified diet that serves as a basal diet for some laboratories, and nutritionally adjusted the diets to compensate for the high levels of creatine or βGPA in the diet (up to 2%). What we did not count on was the profound increase in myotoxicity that simply switching diets produced (*19*). Cerivastatin was profoundly more myotoxic to rats on the AIN diet than on the Purina diet (Figure 2). Fortunately, as the diet switch was a conscious decision on our part and we were concerned that the diet could impact our metabonomics data, we had run the appropriate dietary controls and readily picked up on this finding. Although this diet/toxicity interaction was rather interesting from a mechanistic standpoint, it was totally unanticipated. One might argue that we did nothing more than any reasonable toxicologist would do under the same circumstances. However, unless diet is known to be implicated in the evaluation of the efficacy (or toxicity) of the target, diet may be something that we fail to pay enough attention to when trying to interpret results across laboratories or, harder yet, across departments or institutions.

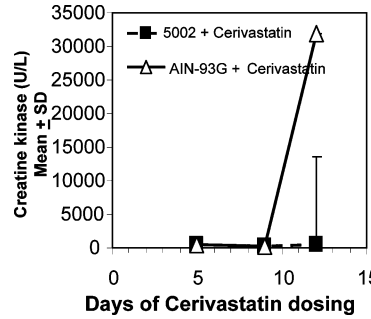

Figure 2. Serum creatine kinase levels (means ± SDs) as an indicator of cerivastatin-induced skeletal myopathy in animals maintained on the Purina 5002 diet (square symbols) or the AIN 93G diet (triangles). Day 5, $n = 6$; day 9, $n = 3$; and day 12, $n = 6$ (except AIN-93G + CVA, $n = 5$). Note that on day 12, CK levels for all AIN93G-fed animals were at the analytical maximum of 32000 IU/L on day 12. Where not visible, error bars are within the symbols. Adapted from *19*.

2.1.2. Example 4: Evolving Models. By definition, if a particular model characteristic is unknown, we can hardly be expected to control for it or take it under consideration in our study design. However, novel findings with regard to models are an almost daily occurrence in the literature. How often do we revise (or potentially discard) our models based on some newly found aspect of its biology? For example, the therapeutic areas of metabolic syndrome/obesity/diabetes is the subject of active research by many pharmaceutical companies. Two recent papers highlight findings of importance to researchers using animal models in those areas. Work by de Wit et al. (*20*) in a dietary-induced obesity model in C57BL/6J mice found that "substantial changes in gene expression in the small intestine, indicating modulations of biological processes, especially related to lipid metabolism. Moreover, we found differential expression of potential signaling molecules that can provoke systemic effects in peripheral organs by influencing their metabolic homeostasis." How often is the small intestine even examined in models of obesity or diabetes?

Another recent paper by Cheng et al. (*21*) examined drug-metabolizing enzymes and transporter expression in the ob/ob mouse, a model frequently used in obesity studies. They found increased mRNA expression of hepatic Cyp4a14, Cyp 2b10, NAD(P)H:quinone oxidoreductase, and sulfotransferase 2a1/2 along with several efflux transporters including Mrp2 and Mrp4 in the ob/ob mice relative to wild type. Concurrent expression of uptake transporters such as the Oatps and Ntcp was decreased. Effects on kidney expression were also noted. These findings, if confirmed, have tremendous implications with regard to the use of the ob/ob mouse as a model, particularly to pharmacokineticists and toxicologists. More importantly, as the authors conclude, if these findings are characteristic sequelea of obesity in general (including in humans), they have broad ramifications across pharmaceutical discovery and development.

These two examples were somewhat randomly selected from the recent literature and are certainly not the only examples of recent work documenting novel characteristics of animal models in use by the pharmaceutical industry. It might be expected that

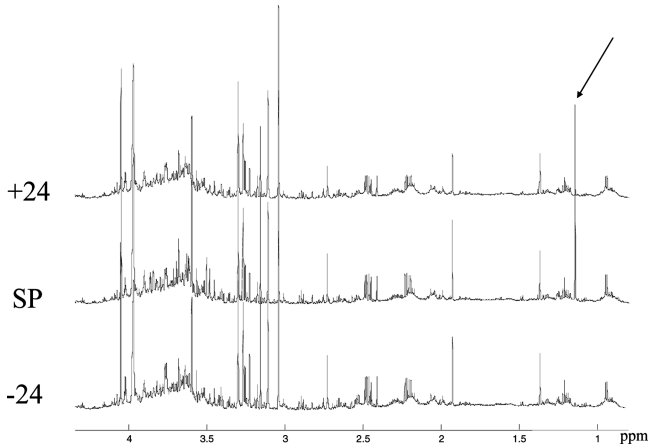

Figure 3. NMR spectrum (600 MHz) from the same untreated guinea pig before (−24) after (+24) and on the day of (SP) receiving sweet potato as a "treat". The arrow points to a prominent resonance peak noted in animals the day of sweet potato feeding. Adapted from *24*; used with permission.

pharmacologists actively involved in such programs would be aware of this work. Less certain is how aware of these findings their partners in other departments may be and how it might affect their data.

2.2. Experimental Methodology. A great deal is known and a great deal has been written on the many variables that can impact study conduct in laboratory animals (*22, 23*). I would guess that most large pharmaceutical companies do a pretty good job of keeping abreast of the latest developments in routine care, handling, assessment, and health maintenance of laboratory animals. Indeed, the problem that the average pharmaceutical scientist faces is that there is too much information to comprehend it all. Not that it is difficult necessarily—There is just so much of it. While most of us went through the dreaded "Standard Operating Procedure (SOP) review" phase early in our employment, digesting the volumes of SOPs of the typical industrial Good Laboratory Practice (GLP) toxicology laboratory and keeping up with the endless updates is not for the faint of heart. This is more than just trivial detail, as the environment and conditions in which we utilize our models are often as important as the species and strain that we are using. In some ways, the sheer magnitude of the institutions in which we conduct our research works against our understanding all facets of a study. Frequently, protocol and study design is done in one group, while routine animal husbandry is performed by another group, actual study conduct may be conducted by a third group, and veterinary support may be supplied by yet another group. While having core centers of expertise is efficient, it makes it difficult for any one person to be on top of every aspect of a study.

2.2.1. Example 5: Impact of SOPs. We experienced a manifestation of this phenomenon early in our work when we were conducting metabonomics experiments in guinea pigs (*24*). After our first study was concluded and the urine NMR spectroscopy was complete, we noted that on particular days, a prominent resonance peak was evident in the spectra of all animals (including controls) that was absent the previous day and the subsequent day (Figure 3). It was clear that this was not a drug effect as it was evident in controls and the incidence and periodicity of the finding suggested that it was more than an anomaly. As it turned out, our SOPs specified that guinea pigs receive "treats" on a regular basis (I guess I missed this SOP in my review...). Furthermore, on the days that the mysterious urinary metabolite peak appeared, sweet potato had been given to all of the animals as a treat. While we never bothered to identify the metabolite, a quick perusal of the web finds that a lot of information is available on urinary metabolites of sweet potato consumption. Who knew?

In retrospect, this problem was potentially foreseeable, but we would have had to put together some fairly eclectic information, namely, that our guinea pigs receive treats, that sweet potato was one of the treats (both facts, which were in our SOPs), and that sweet potato produces a prominent urinary metabolite in guinea pigs. Given the almost infinite number of variations of such study-related parameters that can occur in routine study conduct, it should not be surprising that we occasionally come across what appear to be contradictory or confounding data in our models. The trick is being able to assign these anomalies to their actual root cause.

2.3. Study Control. It is impossible to exactly reproduce animal studies—There will always be some level of compromise with regard to study conduct. Whether it is water, diet, room humidity, handling procedures, veterinary procedures, or any one of the myriad environmental and procedural variables that are part of the daily conduct of a study, it is impossible to exactly reproduce them across studies. In many cases, it is impossible to rigidly fix them within a study. In the vast majority of instances, we know that these things may vary but we choose to ignore them as it seems unlikely that such minor differences will have any impact on the end points that we are measuring. One of the reasons that we run concurrent control groups is so that if any such differences are made manifest we will be able to detect them in our control population and be able to distinguish them from true drug-related effects. The assumption of course is that there is no interaction between these minor variables and the compound under investigation. If such an interaction occurs, the controls may appear unaffected while drug-treated animals produce varied responses dependent on the unanticipated variable. While such interactions are probably rare, they do occur.

3. Recommendations

After reading this, one might conclude I am the ultimate scientific agnostic. After all, what's the purpose of running any animal experiment if we can never really conclude that results mean anything because of all of the underlying variables and assumptions? That is certainly not my conviction. Proper perspective is needed. Despite the examples cited above, the vast majority of work in which I've been involved revealed nothing to suggest that our models were being improperly used or misinterpreted. Doing nothing is a viable option. However, as increasing economic and ethical pressure is placed on the conduct of animal studies, I do believe that we are obligated to at least consider our models and how we use them to ensure that we get optimal results from them. While the need for new animal models for safety testing has been well-recognized (*25, 26*), I would argue that ensuring that the models we do use are as well-characterized, understood, and controlled as feasible is just as vital. Steps to help us do this are not difficult or complicated. Some suggestions include the following.

3.1. Communication. Recognizing communication as an issue may seem a bit hackneyed; yet, it can not be overemphasized. In most industrial and academic settings, there is a wealth of knowledge that lays untapped simply because the need for such knowledge is never communicated. Detailed communications of study protocols (including intent and desires, not just who needs to do what) is an absolute requirement. Better yet, before protocols even come up, informal discussions about what can be done, how it can be done, and what is the best way to do it may bring to the surface all kinds of issues that the time pressure of a scheduled study may suppress.

3.2. "Booties in Room" In the military, there is the expression "boots on the ground", indicating those who are actually doing the fighting. One implication of this term is that these individuals are the most knowledgeable about what actually happens. Similarly, the technical staff that actually has their hands on our animal models ("booties in room") are our most valuable resource about models and what can and has gone wrong with them. These individuals need to be involved and engaged before a study is designed, while it is conducted and while it is interpreted.

3.3. Recognizing Assumptions. We go into every study making some assumptions. We need to recognize what these assumptions are (e.g., diet does not interact with the toxicity of a compound) and acknowledge them when study results vary significantly from the range of anticipated responses.

3.4. Record Keeping. In the rat phenotype example cited above, one reason we were able to resolve this conundrum is that we kept records of which animals we used (with unique ID numbers), which could be traced to specific animal shipments in which we documented supplier and colony source information, and this could be easily done even years after the studies were completed. While this may be fairly standard practice in GLP laboratories, it is not necessarily the case in all laboratories where animals may come from a pool of stock animals, which may be difficult if not impossible to source precisely. Other information such as animal rooms used, diet lots consumed, veterinary procedures employed, etc. may all be important for resolving experimental discrepancies, and this can only be done if adequate records of all salient information are maintained and archived.

3.5. New Technologies. I have emphasized metabonomics in this perspective simply because that is where I have the most experience. Most "omic" technologies (as well as many other types of new technologies) have the capability of revealing otherwise hidden facets of our models. We should take advantage of these tools to more fully characterize our models. In addition to drug-induced effects, such global approaches enable systematic assessment of the genetic, physiological, and pharmacological manipulations that we employ to generate our models. These technologies help us to identify effects that we may not have been looking for but that may be critical for interpretation of data generated with the model.

The fact that we can even discuss such things is a credit to the advancements that have been made across the scientific spectrum over the past decade. After all, I am not suggesting that this "muddle of models" is anything new. It has always existed, but we are now in a position to start doing something about it. It is my opinion that much of what we have attributed to the black box of "normal biological variation" is simply science that we do not yet understand. Although we will never comprehend all aspects of biological variation, as we gain greater understanding of our animals and models, what we currently consider unexplained variability will diminish. This will have the beneficial effect of reducing the number of animals that we need to use within a study as well as reducing the incidence of studies that are not reproducible and findings that we cannot explain.

Acknowledgment. Many thanks to the Metabonomics Evaluation Group (MEG) at Pfizer and the Applied and Investigative Metabonomic Group (AIM) at Bristol-Myers Squibb who have supported my efforts in metabonomics technology over the past 10 years.

References

(1) Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., Van Deun, K., Smith, P., Berger, B., and Heller, A. (2000) Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol. 32*, 56–67.

(2) Olson, H., Betton, G., Stritar, J., and Robinson, D. (1998) The predictivity of the toxicity of pharmaceuticals in humans from animal data-an interim assessment. *Toxicol. Lett. 102−103*, 535–538.

(3) Knight, A. (2007) Systematic reviews of animal experiments demonstrate poor human clinical and toxicological utility. *Altern. Lab. Anim. 35*, 641–659.

(4) Peters, T. S. (2005) Do preclinical testing strategies help predict human hepatotoxic potentials? *Toxicol. Pathol. 33*, 146–154.

(5) Kola, I., and Landis, J. (2004) Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. 3*, 711–715.

(6) Robertson, D. G. (2005) Metabonomics in toxicology: A review. *Toxicol. Sci. 85*, 809–822.

(7) Johnson, A. R., Pavlovsky, A. G., Ortwine, D. F., Prior, F., Man, C. F., Bornemeier, D. A., Banotai, C. A., Mueller, W. T., McConnell, P., Yan, C., Baragi, V., Lesch, C., Roark, W. H., Wilson, M., Datta, K., Guzman, R., Han, H. K., and Dyer, R. D. (2007) Discovery and characterization of a novel inhibitor of matrix metalloprotease-13 that reduces cartilage damage in vivo without joint fibroplasia side effects. *J. Biol. Chem. 282*, 27781–27791.

(8) Skjevrak, I., Solheim, E., and Scheline, R. R. (1986) Dihydrochalcone metabolism in the rat: trihydroxylated derivatives related to phloretin. *Xenobiotica 16*, 35–45.

(9) Crespy, V., Aprikian, O., Morand, C., Besson, C., Manach, C., Demigne, C., and Remesy, C. (2001) Bioavailability of phloretin and phloridzin in rats. *J. Nutr. 131*, 3227–3230.

(10) Starke, A., Grundy, S., McGarry, J. D., and Unger, R. H. (1985) Correction of hyperglycemia with phloridzin restores the glucagon response to glucose in insulin-deficient dogs: Implications for human diabetes. *Proc. Natl. Acad. Sci. U.S.A. 82*, 1544–1546.

(11) Zhao, H., Yakar, S., Gavrilova, O., Sun, H., Zhang, Y., Kim, H., Setser, J., Jou, W., and LeRoith, D. (2004) Phloridzin improves hyperglycemia but not hepatic insulin resistance in a transgenic mouse model of type 2 diabetes. *Diabetes 53*, 2901–2909.

(12) Robosky, L. C., Wells, D. F., Egnash, L. A., Manning, M. L., Reily, M. D., and Robertson, D. G. (2005) Metabonomic identification of two distinct phenotypes in Sprague-Dawley (Crl:CD(SD)) rats. *Toxicol. Sci. 87*, 277–284.

(13) Robosky, L. C., Wells, D. F., Egnash, L. A., Manning, M. L., Reily, M. D., and Robertson, D. G. (2006) Communication regarding metabonomic identification of two distinct phenotypes in Sprague-Dawley (Crl:CD(SD)) rats. *Toxicol. Sci. 91*, 309.

(14) Rohde, C. M., Wells, D. F., Robosky, L. C., Manning, M. L., Clifford, C. B., Reily, M. D., and Robertson, D. G. (2007) Metabonomic evaluation of Schaedler altered microflora rats. *Chem. Res. Toxicol. 20*, 1388–1392.

(15) Boelsterli, U. A. (2003) Animal models of human disease in drug safety assessment. *J. Toxicol. Sci. 28*, 109–121.

(16) Dixit, R., and Boelsterli, U. A. (2007) Healthy animals and animal models of human disease(s) in safety assessment of human pharmaceuticals, including therapeutic antibodies. *Drug Discovery Today 12*, 336–342.

(17) Wells, D. F., Rohde, C. M., Egnash, L. A., Robosky, L. C., Manning, M. L., Roys, M. D., Reily, M. D., and Robertson, D. G. (2007) Muscle creatine depletion exacerbates cerivastatin-mediated toxicity. *Toxicol. Sci. 96*, 53–54.

(18) Reeves, P. G. (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. *J. Nutr. 127*, 838S–841S.

(19) Rohde, C. M., Wells, D. F., Robosky, L. C., Egnash, L. A., Manning, M. L., Roys, M. D., Reily, M. D., and Robertson, D. G. (2007) AIN-93G diet eaxacerbates cerivastatin-mediated myotoxicity. *Toxicol. Sci. 96*, 54.

(20) de Wit, N. J., Bosch-Vermeulen, H., de Groot, P. J., Hooiveld, G. J., Grootte Bromhaar, M. M., Jansen, J., Muller, M., and van der Meer, R. (2008) The role of the small intestine in the development of dietary fat-induced obesity and insulin resistance in C57BL/6J mice. *BMC Med. Genomics 1*, 14.

(21) Cheng, Q., Aleksunes, L. M., Manautou, J. E., Cherrington, N. J., Scheffer, G. L., Yamasaki, H., and Slitt, A. L. (2008) Drug-metabolizing enzyme and transporter expression in a mouse model of diabetes and obesity. *Mol. Pharm. 5*, 77–91.

(22) Schapiro, S. A., and Everitt, J. I. (2006) Preparation of animals for use in the laboratory: Issues and challenges for the Institutional Animal Care and Use Committee (IACUC). *ILAR J./Natl. Res. Counc., Inst. Lab. Anim. Resour. 47*, 370–375.

(23) Conour, L. A., Murray, K. A., and Brown, M. J. (2006) Preparation of animals for research-issues to consider for rodents and rabbits. *ILAR J./Natl. Res. Counc., Inst. Lab. Anim. Resour. 47*, 283–293.

(24) Robertson, D., Beushausen, S., and Pennie, W. (2008) Toxicopanomics: Applications of genomics, proteomics, and metabonomics in predictive and mechanistic toxicology. In *Principles and Methods of Toxicology* (Hayes, A. W., Ed.) pp 293−324, CRC Press, Boca Raton.

(25) Stokes, W. S. (2004) Selecting appropriate animal models and experimental designs for endocrine disruptor research and testing studies. *ILAR J./Natl. Res. Counc., Inst. Lab. Anim. Resour. 45*, 387–393.

(26) Sistare, F. D., and DeGeorge, J. J. (2007) Preclinical predictors of clinical safety: Opportunities for improvement. *Clin. Pharmacol. Ther. 82*, 210–214.

TX800204G