scientific considerations in monitoring and evaluating toxicological research

Edward J. Gralla

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Edited by

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SCIENTIFIC CONSIDERATIONS IN MONITORING AND EVALUATING TOXICOLOGICAL RESEARCH

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Foreword

I have the privilege of introducing you, on behalf of the Chemical Industry Institute of Toxicology, to what we hope will be a stimulating and informative publication. We are gratified by the interest that this subject has aroused and the exceptional cast of authors assembled for this task. The scientific topics to be addressed are fascinating in themselves, important from a practical point of view, and vital with regard to our profession's service to society. In many of the areas covered by our authors, opinions and experiences may differ. There is room for a vigorous and uninhibited presentation of diverse views, which we trust will be forthcoming in ample measure and will help to make reading this book a memorable and fruitful experience.

Another purpose of this Foreword is to broadly address the subject matter in the chapters that follow. There was a time when the scientific community, and especially academia, looked down on toxicity testing as nothing more than mundane routine. Some justification did exist for this attitude. But in many centers–governmental, industrial, and academic–toxicologists have always believed that well-conducted toxicological investigation is research, provided it is imbued by the same spirit of scientific inquiry

as all other research. This is as true today as it ever was. But new factors have entered into the situation. For one thing, good laboratory practice (GLP) regulations are a fact of life, where regulated or potentially regulated products are concerned. This book is intended to look beyond the regulatory requirements of GLPs to scientific considerations that should characterize—in fact, should permeate—the conduct, supervision, monitoring, and evaluation of such work.

I would like to mention briefly certain specific topics that are unlikely to be dealt with in sufficient detail in the chapters that follow. I refer to the hidden imponderables and unperceived factors that exist in laboratory operations. Even though they are capable of profoundly influencing the outcome of experiments, it is often extremely difficult to take effective steps to control these problems.

For instance, there is the question of noise, movement, traffic, drafts, and other forms of stress on the animals. I recall a site visit to an academic laboratory in which I participated; the laboratory was situated in a medical school, where communal facilities existed for animal experimentation. All I can say is that the situation was akin to Grand Central Station or, perhaps more appropriately, Tehran Bazaar when it reopened after the recent revolution. Stress is a much abused concept, but extensive evidence exists for so-called isolation stress of individually housed rats and mice (1,2) with its impact on adrenocortical activity and behavior. Transportation stress may also constitute a factor of unknown dimensions (3). Another problem is that of cross-contamination in the animal facility. By and large, the measures available today permit microbiological cross-contamination to be contained. Such is not always the case with the spread of trace chemical contaminants. The work of Sansone and his colleagues (4–7) at the Frederick Cancer Research Center has established that a very serious problem may be presented by cross-contamination (8), especially of volatile chemicals. Peirce and her colleagues (9) have demonstrated the value of the Ames *Salmonella*/microsome assay for monitoring laboratory contamination by chemical mutagens. Avoidance of cross-contamination within and between animal rooms in a given facility poses both technical and organizational difficulties. It also has an influence on the economics of the entire operation. Cross-contamination can be overcome, and yet, strangely enough, it is a factor that has not received sufficient attention in the past, and to which greater efforts should be directed in future.

In most instances, we are carrying out animal experiments on a compound in order to assess the risk to *people* arising from exposure to that compound. When one surveys the heterogeneity of the human population whose risk is to be assessed, one might well wonder about the overall reliability of the answers that are obtained in animals. Host susceptibility in people arises from a variety of endogenous and exogenous factors, but a particular point to note is the importance of the state of the metabolic

mechanisms that act on the foreign compound. In a penetrating review, Krenitsky (10) recently discussed the evolutionary role played by mixed-function oxidases for lipophilic materials and xanthine and aldehyde oxidases for more polar compounds, including the many heterocyclic toxins that are present in plant foods. These two categories of metabolic mechanisms exist at two levels of activity: constitutive enzyme activity, present in some organs such as the large intestine at very low levels, and induced activity, which applies particularly to the mixed-function oxidases. We are all familiar with the fact that induction by compounds such as phenobarbital enhances the activity of cytochrome P-450, polynuclear hydrocarbons such as 3-methylcholanthrene cause induction of cytochrome P-448, and substances such as Aroclor 1254 bring about mixed induction. The importance of all this is its relevance to organ-specific toxicity, carcinogenicity, and other effects. Hence it is essential to know the metabolic state of the animal as received from the breeder. One way, of course, is to measure the enzymes concerned. Another is to find out the amounts of specific trace contaminants present in the animal. A very interesting paper was recently published on this subject by Eger and his colleageus (11), who found that Swiss ICR mice from California contained no measurable polychlorinated biphenyl (PCB) or polybrominated biphenyl (PBB), within the limits of sensitivity of a method that measured 0.01 ppm. On the other hand, Swiss ICR mice obtained from Michigan had quantities of PBBs averaging 0.068 ± 0.014 ppm. The authors found that the mice from Michigan had a higher incidence of liver lesions when exposed to the anesthetic isoflurane and postulated that liver cancer, which had been produced in the Michigan mice but was not observed in the California mice of the same strain, might have been attributable to this source of contamination.

Having ascertained the state of the animal at the outset, further influences during the course of the experiment should be monitored. The effects of dietary composition are profound. Dietary restriction, especially reduction of caloric intake, promotes longevity (12). It is commonplace nowadays to investigate the levels of a number of likely contaminants in the animal diet (13). Obviously, there are limits to the analyses that can be carried out on a routine basis. What is virtually impossible to control is the composition of the natural—that is, the uncontaminated—components of the diet. In cruciferous plants, for example, there are several compounds capable of inducing mixed-function oxidases, especially aryl hydrocarbon hydroxylase. Four indoles, including indole-3-carbinol, act as inducers, but the existence of other, possibly more active components is suspected in a large number of plant materials. Naturally occurring flavonoids and terpenes can stimulate drug metabolism in liver, as well as in extrahepatic sites in the body. Similarly, there may be inhibitors present in the diet. Then we have the recent discoveries that have revealed the mutagenic potential of flavonoids, particularly quercetin, which is present in most vegetable material (14–16). It

is highly desirable to have some accurate measure of what the diet itself is producing in the way of stimulation or inhibition of metabolic enzymes in key areas of the body throughout the course of the experiment.

I mention these various problems because anyone familiar with current trends in toxicological literature must be aware of the profound effect that such factors might have in influencing the outcome of toxicological studies. We are far from being able to control with complete assurance the various influences that can cause discrepancies between experiments repeated in the same institution and especially in several different institutions. This brings to mind a difference I have had with my colleagues concerning the abandon with which the terms "assurance" and "insurance" have been used interchangeably in discussions of quality control in research. To me, and to Webster, they mean quite different things. Insurance is essentially protection against loss by means of a guarantee of compensation, a contract guaranteeing such protection, or an insurance policy. Assurance is not a legal or a business transaction. It is a state of mind, confidence, inspired by the knowledge that one has done the right thing, taken adequate precautions, applied professional insight, expertise, and experience to assure *oneself* in the first place—not an agency or an inspector or Big Brother—that a job is being done correctly. I put it to you that, while insurance certainly has its place, without assurance on the part of all responsible individuals, insurance alone will not achieve our purpose. I trust that we will all share the feeling that we can apply the lessons learned in this book to the betterment of toxicological research.

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Contents

Chapter 1

Protocol Preparation: Design and Objectives

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DEMANDS FOR A QUALITY RESEARCH PLAN

The complete safety evaluation of a chemical covers an immense area and involves numerous scientific disciplines. Input into the final product—the research report or a publication—is unmatched for scientific diversity. Literally nowhere else in the realm of public-serving, biomedical research are scientific data and opinion collected from so wide a body of contributors and assembled into a single source.

At the receiving end, the practical applications and implications of this work reach or touch an equally heterogeneous audience in industry, medicine, government, academia, and the judicial bodies that write, test, and enforce the law. In short, the givers of this profession—the chemists, biochemists, toxicologists, pathologists, veterinarians, statisticians, etc.—transmit the essence of their efforts and wisdom to the takers—the managers, administrators, regulatory officials, physicians and their patients, industrial hygienists and the workers they protect, lawyers and courts of law, and, of course, that nebulous collection of humans simply called average citizens.

This is an overwhelming concept that seemingly defies simplification. Nevertheless, by retracing the trail this information has traveled from its farthest ramifications in our society back through the offices and laboratories of its origin, we can pinpoint its genesis to the hands of a technician in the animal room of a toxicology laboratory, where the animal and chemical first come together (Fig. 1). Moreover, after reflecting on the demands on and expectations of this network of knowledge, it might be surprising to discover, or perhaps to be reminded, that successes or failures in this entire process rest on the capability and willingness of individuals to do their jobs carefully and consistently each day, for days on end, over long periods. The observers in our cartoon, who are depicted as peering intently at a man and his work, have a vested interest in the outcome. In reality, each one has the legitimate right to ask for answers to the simple question, "How do we know that he has done his job each day, every day, and how can anyone ever really be sure?"

A search through the components of a toxicology program would identify an almost unlimited number of times and places at which the same type of question could be asked. This publication will seek whatever

Figure 1 A primary focus of concern for quality in chemical safety evaluation conceptualized as an animal room located away from constant, direct supervision. Nonetheless, as illustrated, a large body of other professional groups are critically concerned about the outcome.

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