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USE OF SHORT-TERM TEST SYSTEMS FOR THE PREDICTION OF THE HAZARD REPRESENTED BY POTENTIAL CHEMICAL CARCINOGENS*

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EXECUTIVE SUMMARY

It has been hypothesized that results from short-term bioassays will ultimately provide information that will be useful for human health hazard assessment. Although toxicologic test systems have become increasingly refined, no investigator has been able to develop methods which would provide unbiased support for the use of short-term tests in this capacity.

Historically, the validity of the short-term tests has been assessed using the framework of the epidemiologic/medical screens. In this context, the results of the carcinogen (long-term) bioassay is generally used as the standard. However, this approach is widely recognized as being biased and, because it employs qualitative data, cannot be used to assist in isolating those compounds which may represent a more significant toxicologic hazard than others. In contrast, the goal of this research is to address the problem of evaluating the utility of the short-term tests for hazard assessment using an alternative method of investigation.

Chemicals were selected mostly from the list of carcinogens published by the International Agency for Research on Carcinogens (IARC); a few other chemicals commonly recognized as hazardous were included. Tumorigenicity and mutagenicity data on 52 chemicals were obtained from the Registry of Toxic Effects of Chemical Substances (RTECS) and were analyzed using a relative potency approach. The relative potency framework allows for the standardization of data for each chemical of interest "relative" to a reference compound. To avoid any bias associated with the choice of a single reference compound, 14 different compounds were used in separate analyses.

The data were evaluated in a format which allowed for a comparison of the ranking of the mutagenic relative potencies of the compounds (as estimated using short-term data) vs. the ranking of the tumorigenic relative potencies (as estimated from the chronic bioassays). The results were statistically significant for data standardized to 13 of the 14 reference compounds. Although this was a preliminary investigation, it offers evidence that the short-term test systems may be of utility in ranking the hazards represented by chemicals which may contribute to increased carcinogenesis in humans as a result of occupational or environmental exposures.

1.0 INTRODUCTION

1.1 BACKGROUND

Studies in paleopathology indicate that the diseases called cancer appear to have existed even in the prehistoric era (Zimmerman, 1977). Later in history, the diseases of cancer were described by Hippocrates and other Greek physicians (Braun, 1977). One of the first documented observations of a correlation between human life-styles and the development of cancer was that of Ramazzini in 1700, who noted that nuns had a higher incidence of breast cancer than other women. Shortly thereafter, other observations were made which also correlated environmental factors and life-styles (e.g., tobacco snuff, occupation) with the development of cancer (see OSTP, 1985).

In recent times there has been substantial concern regarding an apparent increase in the incidence of human cancers and the causal role of environmental factors (Epstein, 1978; Toxic Substances Strategy Committee, 1980). This correlation has been reinforced by the enormous amount of toxicologic data indicating that chemicals can act as carcinogens in test animals (Weisburger and Williams, 1984; Williams and Weisburger, 1986).

These observations tend to be emphasized by those who wish to highlight the possibility that an increased incidence of human cancers is related to occupational and environmental pollutants (Epstein, 1978). However, the epidemiologic data used to demonstrate an increased incidence, its analysis, and the predictive value of the toxicologic data have all been questioned. Further, other investigators, reviewing the cancer trends in the United States have come to diametrically opposite conclusions (Davis et al., 1981; Doll and Peto, 1981; Peto, 1981).

It is not surprising that such conflicting positions exist. The problem with the analysis of epidemiologic data reflecting cancer trends is that the components of these trends are enigmatic. While this suggests that very rigorous approaches are necessary for the analysis of the data, studies rarely include all relevant issues. These include, for example, the effects of an increased lifespan, changes in smoking habits, increased urbanization, and the decreased incidence of other life threatening diseases (Doll and Peto, 1981; Peto, 1981).

Given the evolving state of the science, and the emotional nature of the issues, it is probable that controversy will continue to characterize the analysis of environmental and occupational agents as causal factors in the production of cancer. Carcinogens and mutagens share an aura, and a societal concern, that is absent from other types of toxicants and the effects that they produce. This appears to be related to the nature of the disease and the hypothesized mechanisms responsible for its appearance.

Nonetheless, despite the controversy regarding the interpretation of the epidemiologic trends and utility of the toxicologic data, in recent decades there has been a virtual explosion in the production of new chemicals. This realization, together with the toxicologic and epidemiologic data (however limited) on these chemicals, dictates that caution and vigilance are in order (NRC, 1984). As noted by Davis et al. (1981),

> (W)here toxicologic data on high-production volume chemicals warrant regulatory intervention, the economic and social costs of waiting for human studies...are inestimable. To wait, renders industrial workers fodder for research, and subjects future generations to potentially irreversible risks. If this view is wrong, regulatory policies can be changed. If it is correct, lives will be saved.

In response to this concern, chemical carcinogenicity and mutagenicity studies have become a standard activity. As might be expected with the intensive increase in testing, an enormous number of chemicals which demonstrate mutagenic and/or carcinogenic activity in

toxicologic bioassays have been identified. However, there is no widely accepted method for the analysis or interpretation of data generated in mutagenicity and carcinogenicity studies.

Many expert committees have convened on this subject, yet the significance of these data (in terms of the public health) is still not well understood. The major problem appears to be that the procedures are still poorly defined and therefore yield inconclusive evidence with regard to human health (Saffiotti, 1978). This can be attributed to the fact that the primary assumptions underlying the study of carcinogens and the evaluation of the data "...have not changed in any fundamental ways since their inception" (Squire, 1984).

The current consensus is that cancers have natural background rates and that these rates can be altered by a variety of exposures. These include biological (e.g. viruses), physical (e.g. ultraviolet light and ionizing radiation) and chemical agents. The mechanisms by which these inciting agents act to produce malignant transformation remain unknown. Factors which act to influence individual susceptibility to these agents include species, age, sex, genetic composition, diet, route of exposure and exposure to other compounds (Slaga, 1980; Weisburger and Williams, 1986). Epidemiologic and toxicologic evidence have also indicated that the production of cancer by radiation (Casarett, 1968) and chemicals (OSTP, 1985; SGOMSEC, 1985) is the end result of sequential multiple biological events. Support for this hypothesis, i.e., a multistage model, consisting of qualitatively different stages--derives from diverse studies (see reviews by Peto, 1977; OSTP, 1985).

The multistage phenomenon has been experimentally observed, using a variety of species, in cancers of the skin, lung, bladder, colon, esophagus, stomach, ovary, mammary gland, liver, thyroid, intestine, and mammalian cells in culture (Slaga et al., 1978; Weinstein, 1985). This process has been generalized to refer to carcinogenic processes in all organs (OSTP, 1985). Although the exact number of stages remains an abstract consideration, it is hypothesized that there are at least two distinct stages: the conversion of a genotypically normal cell to the neoplastic cell (initiation) and the development and progression of the undifferentiated cell type recognized as cancer (promotion) (Berenblum, 1975; Yuspa et al., 1976; Weisburger and Williams, 1986). Some investigators characterize progression (growth of the neoplastic cells) as a third distinct stage (Weinstein, 1985), although this is not as common as the two-stage approach.

In summary, the study of the diseases called cancer has been ongoing for many decades; however, attempts to delineate the mechanisms were started much more recently. The disease is complex, progressing through multiple pathological stages, and each stage can be subdivided. Some experimental evidence has been interpreted to indicate that the progression through these stages can be affected by different classes of compounds. The observed effects can also be influenced by a variety of modifying factors. Additionally, in contrast to conventional toxicologic problems--which involve assessments of agents which generally have identifiable thresholds--there does not appear to be a measurable dose dependent threshold for many types of physically or chemically induced cancers (Yuspa and Harris, 1982; OSTP, 1985; Weisburger and Williams, 1986).

These issues clearly distinguish the diseases of cancer from most other types of pathologies. This has forced the development of very specialized methods for the evaluation of the danger associated with exposure to a compound. The results of these studies are conceptually integrated in the "risk assessment" of that compound. However, the later phase of activity is routinely constrained by problems of terminology, particularly with regards to the difference between hazard and risk. From the perspective of the toxicologist and epidemiologist, the ambiguities in terminology tend to be accompanied by applications of data which are inadequate, inappropriate, and misleading. Unfortunately this produces assessments which, like the "matador's cape," attract much attention; however, lacking scientific credibility, they have little substance (Gillette, 1985).

The purpose of this project was to demonstrate a method by which toxicologic data could be used in a "risk assessment" while recognizing the limitations of the data. Hence, in the context of this document, the term risk (probability of an adverse health effect in humans as the result of exposure to a compound) will be reserved for estimates generated using epidemiologic studies; the term hazard (determination of whether a compound is causally related to the production of an effect in an experimental setting) will be used to refer to conclusions based on toxicologic data.

1.2 RESEARCH QUESTION AND OBJECTIVES

Can results from short-term tests be used to predict the hazard of a compound as estimated using the carcinogen bioassay? To address the research question the following steps were taken:

1. A review of the underlying assumptions and subsequent limitations of toxicologic and epidemiologic data was performed. The impact of these limitations on the use of toxicologic data for regulatory purposes was specifically assessed.

2. Alternative methods for the analysis of the toxicologic data were examined and a suitable method was chosen. The technique chosen, a relative potency concept, is very versatile to address the research question.

3. An appropriate source of information was identified (e.g., a compendium of quantitative toxicologic information).

4. The relative potency concept was modified to allow for an analysis of the data which minimized assumptions and eliminated the need for untestable models.

2.0 LITERATURE REVIEW

This chapter reviews methods currently employed to assess human cancer risk associated with exposure to chemicals. The chapter is divided into four major parts. The first part is a comprehensive review of the issues which distinguish the toxicology and epidemiology of cancer from other pathologies. The second part concentrates on the various methods used for the application of the toxicologic data. The discussion includes strategies being explored by regulatory agencies to use toxicologic data for "risk assessment" as well as approaches that have been developed to "validate" the short-term tests (i.e., test for correlations between the data generated using short-term bioassays and data obtained from chronic bioassays). The third part focuses on an alternative method to analyze toxicologic data used to assess the potential carcinogenicity of a compound. The fourth and final part is a critical synthesis of the previous literature.

2.1 REVIEW OF ISSUES WHICH DISTINGUISH CHEMICALLY INDUCED CANCER FROM OTHER TYPES OF TOXICITY

2.1.1 Conceptual Issues in the Toxicological Evaluation of Chemical Carcinogenicity

Toxicology is defined as the evaluation of the harmful actions of chemicals on biologic systems (Loomis, 1978). It is generally believed that most toxic chemicals share certain properties; Saffiotti (1977) has termed these properties "terminal toxic effects" and the study of these effects "traditional toxicology." According to Saffiotti (1977), the properties include:

1. The rapid appearance of the effects.

2. A clear correlation between the intensity of the pathology and the magnitude of the exposure.

3. A manifestation of toxicity that can be corroborated with altered functional products, degenerative changes, or death of the target cells.

4. A dose below which no harmful effects are observed (i.e., a threshold).

5. Observations indicating that the damage is reversible.

6. Observations suggesting that the intensity of the response over time is a reflection of the organism's capacity to reduce the effective concentration of the chemical at the site where the effect is observed.

In practice, the threshold dose represents a dose below which no effect is observed [No Observed Effect Level (NOEL)]. An acceptable dose for human exposure is then determined by simply applying an arbitrary "safety factor" to the NOEL (Klaassen, 1986). This approach has been euphemistically described by an anonymous source, cited by Saffiotti (1977), as having said: "Find a no effect level in animals, divide by 100, and pray." Recent work has been performed to provide a greater credibility to the safety factors (Dourson and Starra, 1983).

The biologic effects recognized under the rubric of carcinogenesis, on the other hand, refer to the long-term effects from chronic exposure to an agent. These effects are of insidious onset and can appear long after the exposure to the given agent has ceased; this lag time is defined as the induction time or latency period. The major problem in categorizing these effects is that there are no known early indicators of the potential ramifications of the exposure and that the endpoint is not seen in all individuals exposed to the agent.

Even advances in biochemistry and physiology have not provided researchers with the capacity to elucidate the molecular complexities of what is recognized as biologic transformation. Hence, an operational definition has developed to incorporate the observations associated with the morphological consequences of transformation. Given a phenomenon so poorly defined, one could easily argue that the extenuating circumstances which affect the natural history of the disease could be "defined" as carcinogens, even though it is believed that these circumstances act to modulate processes in the biological assay systems employed. In laboratory experiments, these factors have been demonstrated to include a wide variety of stressors, including for example, cage overcrowding, noise, age, diet, space restrictions, and other factors which stress the biology of the exposed test animal (Gori, 1980).

More accurately, it is generally agreed that several events are needed at the molecular level to produce cancer, and although a number of hypotheses have been proposed and investigated, the mechanisms are largely unknown. These events modify the genome and/or molecular control mechanisms in target cells such that these cells give rise to progenies of permanently altered cells. The growth of these progenies is what is observed as the toxic event. The significance of this is that the observation occurs at the morphological level, does not derive from the same cells that were exposed to the toxic agent, and is recognized only by the proliferation of populations of altered cells (IRLG, 1979).

The study of these events obviously entails major differences from the studies described for the area of "classic toxicology," where a significant characteristic of the pathology is self-limiting toxic effects. As an alternative, Saffiotti (1977) has referred to the study of these self-replicating toxic effects, or errors in replication, as the "new toxicology." These effects are characterized by:

1. The expression of adverse health effects is delayed (e.g., latency period between the exposure and the observation of the effect)[2].

2. The frequency of the expression of the injury (number of individuals with tumors) in the exposed population appears to be dose dependent.

3. The intensity or severity of the injury (number of tumors per individual) appears to be independent of dose.

4. The manifestation of toxicity is observed as a proliferation of a new (unexposed), altered cell population.

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[2]The "observation of the effect" refers to the growth of the neoplastic tissue, not necessarily an absence of a "signature" of the exposure. This can be attributed to the subclinical or "silent" events which characterize the latency period (e.g., unmeasured molecular events). Hence, a critical feature of this distinction is that "latency" may actually represent a failing of diagnosis, both experimentally and clinically.

5. Theoretically, the actual critical molecular injury may be very limited (i.e, limited to a few cells or molecules) and is usually not detectable using available methods.

In conclusion, these issues limit the definition to an operational one, for the intensity of the response (number of tumors per individual) appears to be susceptible to a number of factors which have the capacity---via unknown mechanisms---to affect the conditions of the host organism. These factors are recognized as ranging from "modulating" agents (such as stress), to dietary factors, to viruses (Williams and Weisburger, 1986). Hence, in general, it must be understood that neither the disease cancer nor the term carcinogen is well defined. Therefore, allusions to malignant transformation should be recognized as nonspecific and referring to a variety of diverse diseases caused by many distinct and specific determinants. While these issues distinguish malignant transformations from other types of pathology, the perspective is still compatible with a multifactorial perspective of disease (Weiss and Liff, 1983).

2.1.1.1 Toxicologic methods of testing for chemical carcinogens

In 1958, the Delaney Amendment (Public Law 85-929, 85th Congress, HR 13254) to the Food and Drug Act was adopted and inadvertently opened a Pandora's Box. Although relatively short, it is possible that no other single piece of legislation has caused such great turmoil in the health and science policy of this country. In brief, the amendment simply states that no material demonstrated to be a carcinogen in any species, at any dose, by any route is permitted to be a food additive.

The amendment represented a translation of the no-threshold concept used for ionizing radiation to the realm of chemical carcinogens. At the time of the amendment's passage, the notion of chemical carcinogenicity was still obscure; chemical carcinogens were considered to be anomalies in man's environment. Since that time, using a generalized notion of the Delaney Amendment as the guide, major efforts have been dedicated to the study of chemical carcinogens (Albert, 1980; OSTP, 1985). These activities have produced a variety of techniques which are used to assess the potential carcinogenicity of a chemical.

2.1.1.2 Short-term tests for potential carcinogens

It is well recognized that radiation and some chemical agents are able to induce permanent changes in the genotype of a cell. These changes, which may also occur spontaneously, are classified as mutagenesis (occurrence of sharply localized change in base-pairs), aneuploidization (gain or loss of one or more intact chromosomes), and clastogenesis (chromosomal breaks resulting in gain, loss, or rearrangements of pieces of chromosomes) (Thilly and Call, 1986).[3] Bioassays which measure the occurrence of these events *in vitro* (microbial and cellular test systems) can therefore be grouped by endpoints as follows (Interdisciplinary Panel on Cancer, 1984; OSTP, 1985; Thilly and Call, 1986):

1. Biochemical assays---test for DNA breakage, adduct formation, strand breakage, prophage induction, and DNA repair.

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[3]For simplicity, the term "mutation" will be used as a broad reference to denote all chemically induced modifications of the genome.

2. Mutagenicity assays---test for forward and reverse mutation, altered DNA, altered gene products, or altered cellular behavior.

3. Assays for general DNA damage---characterize aneuploidy, structural aberrations, micronuclei, and sister chromatid exchange.

4. Assays for neoplastic transformation---characterize altered growth patterns and altered cellular morphology.

Given the lack of understanding of the underlying mechanisms responsible for the production of these types of damage (for either "natural" or exogenously induced effects), the tests are used to provide empirical evidence of the potential for chemicals to act as human mutagens.

The theory underlying the use of these tests is that cancer is the result of DNA damage. The hypothesis that heritable alterations in the genetic material could result in malignant transformation was first posed in the early part of this century (Boveri, 1929). However, Boveri's thesis---which posited that tumors developed as the result of somatic mutation---must be interpreted using the description he offers of his work.

In the seminal publication The Origin of Malignant Tumors (1929), Boveri clearly refers to "mutations" observed using a light microscope. This would indicate that Boveri was alluding to chromosomal abnormalities---what is now categorized as aneuploidization---as the causal factor. This observation has been generalized, and the "somatic mutation theory of cancer" has been extended to include all chemically induced modifications of the genotype (OSTP, 1985). In accordance with the augmented "somatic mutation theory of cancer," short-term bioassays have been designed to test or screen for potential carcinogens using a variety of genetic effects in "simple" (*in vitro*) and "limited" (*in vivo*) test systems. The assays---approximately 100 tests are now available---are performed using diverse protocols, a wide variety of test conditions, and, as discussed, can include studies ranging from isolated DNA to observation of cells temporarily implanted into a whole animal (host-mediated assays) (Hollstein et al., 1979). In general, the most commonly used assays employ bacteria or cultured mammalian cells (Anderson, 1981; Brusick, 1983; ILSI, 1984a).

The major advantages of this type of assay system are that it is neither as time consuming nor as expensive as the whole-animal bioassay. Also, because of this type of assay's relative simplicity, such assays can be directed at a variety of endpoints, extending from the study of mutagenicity in bacteria to the mechanisms which induce neoplastic transformation in mammalian cells in culture. Alternatively, the limitations of this type of assay system derive from the fact that the results of these tests are not the production of malignant tissue (a response of the organism) but of alterations at the molecular, cellular, or multicellular level.

2.1.1.3 Assumptions and confounding issues associated with the short-term bioassays

The basis of the short-term tests is the theory that cancer is the result of DNA damage.[4] This theory suggests that those agents, that have the ability to produce somatic

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[4]Obviously, there are significant differences in the DNA of different life forms. However, since all but the most basic life forms (e.g., "slow viruses") have their biologic properties encoded in DNA, it is generally assumed that what causes a mutation in one organism can cause a similar event in another organism. This reasoning is employed as the justification for the use of simple life forms, such as bacteria, as acceptable surrogates for

mutations, may also be carcinogens by acting on one or more of the stages leading to malignant transformation. Attempts to validate the short-term test systems using "known carcinogens" have produced an apparently high correlation between mutagenicity and carcinogenicity (Brusick, 1983). This correlation appears to support the "somatic mutation theory of cancer" and the use of the short-term tests as "screening" tools.

However, upon closer examination it becomes apparent that the use of this type of technique to validate the short-term tests is inherently biased. The bias derives from the fact that few chemicals have demonstrated adequate evidence of a lack of carcinogenic activity (IARC, 1982; Tomatis et al., 1982). Therefore, due to a limited number of alternatives, it is inevitable that the sample of compounds used to evaluate the correlations will contain a high proportion of carcinogens.

The implication of these observations is that the correlations calculated using this approach may be spuriously high as the result of the large proportion of carcinogens in the test sample; the correlations may be reduced when the sample contains compounds which have been adequately demonstrated to be noncarcinogens (Tomatis et al., 1982). The conceptual significance of these investigations is also limited by the tendency of some investigators to confuse the criteria of screen sensitivity (ability of a test to detect carcinogens) and predictive value (proportion of carcinogens among the substances tested which yield positive test results) (Cooper et al., 1979).[5]

Other confounding issues in the interpretation of these results are that some compounds do not exert their carcinogenic effects via an interaction with nucleic acids and that most of the short-term tests cannot take into account the complexity of the whole organism. Not surprisingly, both positive and negative results are usually encountered when one reviews a battery of short-term tests, making it difficult to draw firm conclusions about the properties of the agent studied (ILSI, 1984a).

2.1.1.4 Conclusions regarding the application of the short-term test systems

Although the short-term test systems appear to be effective at measuring their respective endpoints, it is not known what the relevance of these results is in terms of the phenomenon of neoplasia (ILSI, 1984a). Hence, lacking corroborating data on their carcinogenicity from in-vivo or epidemiologic investigations, judicious interpretation of the results from the short-term test systems is recommended. In recognition of these caveats, some investigators have proposed that while the short-term bioassays should continue to play a role--and be further researched--to be prudent, they should be employed only for suggestive evidence of carcinogenic potential (IRLG, 1979; Squire, 1981; NRC, 1984; OSTP, 1985).

2.1.1.5 Long-term or *in-vivo* bioassays

As with the short-term tests, the tenets of the long-term bioassay that is currently used to assess carcinogens were developed in the early part of the century. The technique was developed in order to determine if a pharmacologic agent was capable of producing a particular effect. If the effect was produced, the bioassay served as a means to quantify the potency of the drug and/or the associated toxicity from responses in test animals. The bioassay was particularly effective when working with crude materials--i.e., different samples with an unknown content of the active agent (Shimkin, 1977; Goldstein et al., 1974).

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mutation in human somatic cells.

[5]This topic is also reviewed in Section 2.2 of this chapter.

The original application of the bioassay approach to carcinogenesis focused on the use of carcinogens as tools---i.e., as a means of producing cancer in the test animals. This provided a convenient means of studying the biologic mechanisms and processes of the disease. In order to manipulate the desired outcome, strains of test animals were inbred to increase or decrease the probability of their developing certain types of cancer (Berenblum, 1974).

It must be emphasized that the investigators responsible for the development of these bioassay methods did not intend for them to be used as a means of screening chemicals for potential carcinogenicity (OSTP, 1985). As noted by Shimkin (1977), the original use of the chronic bioassay for this pursuit was simply a means of providing a temporary, albeit crude, method of analysis until more definitive procedures were available. A detailed history of the use of the chronic bioassay for carcinogenicity studies can be found in Weisburger and Williams (1984).

Protocols for the use of the long-term bioassay for carcinogenesis studies have been published by a variety of national and international organizations, including, for example, the National Cancer Institute (NCI) (NCI, 1976) and the International Agency for Research on Cancer (IARC) (IARC, 1980). Also, an updated version of the NCI program has been authored by the National Toxicology Program Board of Scientific Counselors (NTP, 1984).[6] Each of these documents reviews the spectrum of assumptions that are necessary for the evaluation of results obtained using this bioassay system. The protocols are designed so that, as with any well-designed laboratory experiment, they attempt to provide the investigator with the capacity to "isolate" the factor hypothesized to produce the changes recognized as cancer. In brief, when sufficient evidence of an increase in tumors is found in the treated animals relative to concomitant controls, the substance is labelled an animal carcinogen and a potential human carcinogen.

However, even those investigations which use sanctioned protocols are beset with substantial obstacles. These derive from the time necessary to complete the experiment (the assay can take years to complete), the cost of the experiment (currently in excess of a million dollars per chemical), and a wide variety of other factors which can influence the outcome. Further, from a technical perspective, the long-term bioassay is a logistical nightmare: some predetermined number of animals must be kept free of disease or any other risk factor for a number of years, the animals' food and water must be free of potentially confounding compounds,[7] animal husbandry staff must follow strict regulations, and other management problems (which are discussed in detail in the aforementioned protocols) must be resolved.[8]

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[6]For more references on the published guidelines for the design and conduct of the long-term bioassay, see OSTP, 1985.

[7]For example, Schoental (1978) has noted that variations in the rates of "spontaneous" tumors in the control animals might be attributable to the contamination of food with carcinogenic mycotoxins.

[8]The myriad of factors which can influence the outcome of the bioassay is so great that some investigators feel that the use of concomitant controls is not sufficient and thereby advocate the use of "historical controls" as well (NTP, 1984).

It should be noted that the term "historical controls" is a generic phrase used to refer to data obtained over the past decade on the tumor incidence in untreated animals. "Historical controls" are particularly useful in situations where there are small differences between the incidence of treated and control groups, especially if the rates can be demonstrated to be within the rates observed in other experiments (OSTP, 1985).

Despite the use of rigorous protocols and appropriate controls, the assay can still yield statistically inconclusive data. To address this impasse, statisticians calculate probable false negative and false positive rates for experiments; this introduces a quantifiable element of uncertainty to any conclusions (Fears et al., 1977). Hence, from a quantitative perspective, the long-term bioassay appears to be a crude, costly tool and highly prone to error.

2.1.1.6 Assumptions and confounding issues associated with the *in-vivo* bioassays

In accordance with the issues discussed in the preceding section, some scientists contend that the chronic *in vivo* bioassay yields data of limited value for risk assessment, both quantitatively and qualitatively. The problems most frequently cited include: the assays were developed as a method to study the processes of carcinogenesis; the results are simply empirical observations; there tends to be unaccountable variability in the background rate of disease in the control population; and the procedures are long, complex, and error prone. These issues have prompted some investigators to claim that this type of testing is probably most useful for the study of biochemical mechanisms but has limited utility for human hazard assessment (Gori, 1980; Squire, 1984).

Despite these limitations, the demand for the testing of potential carcinogens has increased enormously. This has encouraged the involvement of investigators and laboratories which, for a variety of reasons, produce results that are highly variable, and, at times, of dubious quality (Saffiotti, 1977). The lack of uniformity in study designs and interpretation of study results has contributed to the controversy regarding the utility of the bioassay.

For example, a panel of experts reviewing the data on a given compound may disregard results that do not meet their requirements. In response, the Task Force of Past Presidents of the Society of Toxicology issued a statement regarding the use of animal data for hazard evaluation. With particular emphasis on the "flawed study," the task force stated that it was inappropriate to dismiss data simply because the investigators of the data did not meet the current standards of experimental practice for the study of potential carcinogens. Rather than an outright rejection of such data, it was suggested that all data are "...entitled to some weight, but how much is a matter of scientific judgment" (Task Force of Past Presidents, 1982).

Thus, the long-term bioassay is fraught with difficulty and controversy. The assay tends to yield data which require extensive, careful analysis before a compound can be designated as a carcinogen in test species. It is believed that this process is greatly expedited when the studies conform to "recognized" protocols, whereas the scientific merit of those studies which use less rigorous protocols is, of course, more difficult to evaluate (Saffiotti, 1977). Nonetheless, regardless of how well the experimental protocol conforms to existing standards, most data can be used in some capacity (Task Force of Past Presidents, 1982).

The importance of data derived from the long-term bioassay derives from three major factors:

1. The use of human beings for the testing of potential carcinogens, even at minuscule concentrations, is grossly unethical.

2. Quantitative epidemiologic evidence, although widely held to be the most conclusive type of evidence, is generally unavailable for the myriad of chemicals being manufactured.

3. Short-term tests, whose usefulness and utility will probably continue to grow, have not yet demonstrated sufficient predictability to be employed as the sole determinant of cancer in humans.

The significance of the long-term bioassay is further amplified, and possibly exaggerated, by the current tendency to attempt to convert or extrapolate the results to the human population. These interspecies conversions, some of which will be reviewed, involve a vast number of currently untestable assumptions. The inability to verify these assumptions can result in regulatory decisions that are lacking in scientific justification. Representative assumptions include:

1. **Interspecies differences.** It is often assumed that only minor differences exist between species. Yet, it is well recognized that the physiological and biochemical traits of one species (even strains) can be vastly different from that of another (Langenbach et al., 1983). This is particularly significant when using homozygotic test strains in which certain factors are purposefully "bred in" (to enhance the sensitivity of the test strain), causing other factors (potentially unknown) to be "bred out." There is no way to predict the total effects of directed reproduction.

   The overall effect of selective breeding is unknown and could render the organism more susceptible to certain agents and less susceptible to others. The implications of this assumption must then include the recognition that a substance may be carcinogenic in some species (or strains), yet harmless for others. Given these observations, it is plausible to expect that the susceptibility of humans may be similar to or widely divergent from what is observed in the test species (Purchase, 1980b; Gillette, 1985).

2. **Dose considerations.** This issue has caused enormous controversy (ILSI, 1984b; Haseman, 1985). In order to elicit the development of the chronic effects in a reasonable period of time and ensure the statistical credibility of the results, extremely large doses are usually employed. These exposures are much higher than would be encountered in the generalized "natural" setting. The doses are calculated as fractions of the "maximum tolerated dose" (MTD).

   The MTD is generally defined as the maximum dose to which an animal can be exposed for a lifetime without the production of significant impairment of growth (the acceptable limit is generally 10% variation of expected weight), reduced longevity (other than that due to neoplasia), or other types of overt toxicity[9] (IRLG, 1979; ILSI, 1984b). However, this definition is widely disputed, leading to confusion in dose selection. Debate seems to be focused on the level of acceptable toxicity for a dosage to be sanctioned as the MTD (ILSI, 1984b; Haseman, 1985).

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[9]A number of issues can impact the conceptual value of this approach. For example, a compound could produce a profound distortion in the biochemistry or physiology of a particular organ. In turn, this could have enormous ramifications on the pharmacokinetic and pharmacodynamic properties of the substance in question. This effect may not be evident using whole-body weight as the sole index of toxicity.

Advocates maintain that the use of the MTD is necessary to overcome the weak detection power of the experiments, whereas critics address their concerns to the notion of biologic plausibility. For example, since the MTD is administered in the proximity of the LD50, it may produce subclinical toxicity which could influence the response of the organism to the chemical under investigation (Weisburger and Williams, 1984; Haseman, 1985). Hence, the observations at or near the MTD may not be qualitatively relevant to the potential toxicity associated with low-dose human exposure.

3. **Extrapolation from high to low doses.** The use of this approach infers that responses occurring at high doses (near the MTD) can be employed to predict responses at the lower dose levels. The technique is employed because experimental evaluation of events at low levels of exposure are usually not possible due to statistical considerations. These are related to the inherently low sensitivity of the chronic bioassay. To compensate, very high doses are used and mathematical models are employed to extrapolate the experimental results to doses and responses outside of the range of that which is observed in the experimental setting (Munro and Krewski, 1981; OSTP, 1985).

   However, dose-dependent variations in the pharmacokinetics and pharmacodynamics of a compound are well recognized. Studies on a variety of compounds producing different effects have clearly demonstrated that as the dose varies there are possible concomitant variations in the biochemical and physiologic status of the animal. These dose-dependent alterations can modify the biologic fate of the chemical and consequent biological effects (Goldstein et al., 1974; Gehring et al., 1977; Reitz and Watanabe, 1983; Ames et al., 1987).

4. **Interspecies conversions and comparisons.** The basis of this assumption is that a nonhuman mammalian species can be used, in an experimental setting, to categorize a chemical as a potential human carcinogen.[10] It also assumes the validity of applying these data to estimate the human cancer risk associated with exposure to the chemical. The quantitative application of experimental data to the human population incorporates a "...range of doses from the barely tolerable to the barely measurable and a radical change in species" (Paddle, 1980). These clearly represent gigantic assumptions, whose application requires a "leap of faith."

5. **Mathematical modeling.** Several different models have been proposed to extrapolate data from the region of observed results in an experimental species to predict cancer rates in the human population as a consequence of doses which are frequently barely measurable, and, at times, unknown. Unfortunately, current knowledge does not dictate a specific model, nor does the type of evidence obtained from the long-term bioassay generally allow for discrimination between different models, even in the range of observable responses (SGOMSEC, 1985). This is because the data generated in these experiments tend to be comparatively crude (i.e., a carcinogenicity

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[10]As noted, it is not clear why some test species develop pathologic responses after exposure to a compound whereas others do not. The critical feature of this assumption is that it is presumed that humans will respond similarly to the test species which developed the pathologic tissue. In reality, it is also possible that humans may be more comparable to the animals which are less sensitive.

bioassay is usually limited to two or three doses) (NRC, 1983; ILSI, 1984b). Further, positive responses may only be observed in the group exposed to the highest dose. Protocols of this type yield maximal information on the carcinogenic potential of a compound (i.e., they maximize the sensitivity of the study) but offer very little information about the "true" shape of the dose-response curve (NRC, 1983; Downs, 1985).

In practice, multiple models can fit the observed data equally well yet lead to low-dose extrapolations which vary by several orders of magnitude (Brown and Kozial, 1983; Downs, 1985; Hoel, 1985; Jones et al., 1985). When the physiologic mechanisms and/or pharmacodynamics of the compound are known, models can be developed to incorporate this information. However, by definition, a model is limited in its context and thereby cannot be expected to incorporate all of the parameters which may affect a biologic system. Other problems include: the lack of a "weighting" factor which can reflect the rigor of the data (e.g., how well a recognized protocol was followed), that most models are limited to using data generated in a single chronic bioassay, and the incorporation of somewhat arbitrary assumptions (Munro and Krewski, 1981; Brown and Kozial, 1983).

Thus, although the *in vivo* studies serve as the primary surrogate for the human population, any attempt to employ the data beyond the realm of experimental verification must be seriously questioned (Cornfield et al., 1980; Munro and Krewski, 1981; Brown and Koziol, 1983). Until more is learned about the mechanisms of chemical carcinogenesis, high-to-low dose extrapolation, and interspecies conversions, methods employed to model these variables will have severe limitations (NRC, 1983).

6. **Thresholds.** Classical toxicology assumes that all chemicals have a dose below which one cannot demarcate an effect (threshold dose). The notion is much more complex for chemically and physically induced carcinogenesis, and opinions are divided. Scientists favoring the absence of a threshold cite the fact that, in theory, a single molecule of a chemical may have the capacity to induce an irreversible, self-replicating lesion, "initiated" as a mutation in a somatic cell. Support for this view is also derived from measures of experimentally induced cancer which indicate that the incidence is proportional to dose and a fixed power of time (Purchase, 1985b). Those in opposition to this argument present information on repair mechanisms which may be able to negate the genotoxic effects, especially at very low doses (Gehring and Blau, 1977; Downs and Frankowski, 1982). However, the relationship of DNA repair and a possible threshold cannot be experimentally substantiated (OSTP, 1985).

Experimentally, as seen in all bioassays, NOELs are also observed in the carcinogenesis bioassays. Investigators who believe that carcinogens represent a finite risk at all dose levels ("no-threshold") maintain that this observation simply represents the limits of detection for the bioassay. In turn, since most bioassays cannot detect statistically significant increases in risk below a 5% to 10% increase above the risk to controls, these NOELs are not believed to demonstrate a true threshold, i.e., they are not of biologic significance (Haseman, 1985).

Actually, and more pragmatically, the notion of thresholds must be viewed in the context of every other biological parameter--varying from individual to individual. Also, given the well-recognized biological effects of factors such as diet, age, illness,

and stress, it cannot even be assumed that an individual's threshold will be constant (Bingham, 1971). To attempt to compensate for these possibilities and meet the needs of regulatory concerns, the "minimum of all thresholds" is estimated in the form of a theoretical population threshold. The problem is thereby converted from a biological problem to a statistical one. This has been a source of great debate, and, as with the other assumptions, has defied experimental verification[11] (Rall, 1978; Cornfield et al., 1980; OSTP, 1985).

Therefore, in the interpretation of experimental data it is important to distinguish between an apparent or practical threshold (observed in a population) and a true biological or pharmacological threshold (where no individual demonstrates an effect below a given dose). Although the population-based threshold is used for the development of regulations, in terms of the individuals constituting the human population the question assumes a more philosophic tone, to wit, " . . . whose threshold and when?" (Rall, 1978). Even if a measurable threshold existed for individuals, a single threshold would probably not be applicable to entire populations (NRC, 1983). As such, the threshold concept does not appear to be a useful construct for the assessment of carcinogenic hazard (Bingham, 1971).

7. **Potency.** Again, in classical toxicology (effects other than carcinogenicity), the concept of potency refers to dose-dependent effects, i.e., per individual, the intensity of the induced effect appears to be strictly determined by the intensity of the exposure. However, in studies of neoplasia, the intensity of the response (i.e., the number of tumors per animal) appears to be independent of the intensity of the exposure (Saffiotti, 1977).

   Factors known to influence the severity of the individual's response are very diverse and include the age, sex, individual genetic differences, test strain, species, diet, dose rate, route of administration, vehicle, other exposures, and environmental conditions prior to, during, and after the exposure (OSTP, 1985). Hence, when one considers that a strict definition of a carcinogen is still lacking and the enormous variety of factors which can influence the results of the chronic bioassay, it is probably prudent to only employ a carefully qualified concept of potency.

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[11]The one investigation (euphemistically referred to a the "megamouse study") that was large enough to scientifically address the issue of a biologically measurable population threshold produced equivocal data; at low-dose exposure to the genotoxic carcinogen acetylaminofluorene (AAF) the bladder demonstrated an apparent threshold, whereas the liver did not (Hughes et al., 1983; Staffa et al., 1979). The significance of these observations--i.e., the lack of resolution of low-dose responses even in an experiment designed to evaluate them—cannot be overemphasized. However, given the idiosyncracies of the chronic bioassay, even if the existence of a population-based threshold for chemically induced neoplasia was a measurable event, it could be argued that the observation was limited to the experimental context in which it was observed (Gillette, 1985; Cumming, 1985; Gori, 1980).

2.1.1.7 Conclusions regarding the application of the *in vivo* test systems

Despite the large investment in human, animal, and fiscal resources, the long-term bioassay still produces results of uncertain quantitative and qualitative significance to humans. While the use of the bioassay provides an enormous amount of data, a lack of knowledge of underlying mechanisms limits the capacity to understand and generalize the results. Although experimental data can be used with some assurance in the qualitative assessment of hazard, methods are still lacking which would justify making scientifically acceptable, direct quantitative extrapolation of risks using experimental studies (Bartsch et al., 1982; OSTP, 1985; Ames et al., 1987).

For reasons that remain unclear, none of the bioassays or statistical models used to summarize and "extrapolate" the data are chemical specific. Further, the use of different models produces widely divergent estimates of risk for a given chemical. The divergence in the estimates may be a reflection of the mathematics rather than of the biologic processes being modeled. However, until more information is available regarding the etiology of the disease, these issues cannot be resolved.

The magnitude of these uncertainties are aptly demonstrated by the debate engendered in the scientific community by the use of these models, yet ". . . few relevant data are available to the proponents of either side" (Tomatis et al., 1982). As noted by the Task Force of Past Presidents (1982), the failure to understand these underlying limitations can lead to conclusions and subsequent decisions which can become accepted as dogma and lead to serious errors in the decisions made about a compound.

2.1.2 Validity and Biases of the Toxicologic Bioassay Test Systems

Given the current mode of testing used to evaluate the carcinogenic potential of a xenobiotic, one must recognize that the recommended short-term and chronic bioassays, although very specialized, tend to have caveat-laden interpretations (i.e., limited internal validity).[12] Consequently, it has been speculated that (despite the sophistication of the bioassays) the bioassays have a limited capacity to be used for generalized applications (i.e., external validity) (Somers, 1982).[13] Even if the basic methodological problems of the studies are overcome, limitations remain because experiments undertaken with the strictest protocols

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[12]The internal validity of a study refers to inferences about the experiment, using information from the study design. For example, if a toxicologic experiment is properly managed, in theory, the investigator can assume that the observed responses (e.g., tumors) are directly related to the chemical of interest (e.g., the potential carcinogen). However, as noted, carcinogen bioassays are plagued by a number of confounding variables which can influence the outcome. (Weisburger and Williams, 1984; Gori, 1980).

[13]The term external validity is used to refer to applications beyond the immediate implications of a given experiment. Conceptually, this is the basis for generalizing study outcomes. For these investigations, external validity issues include, for example, (1) the use of the cancer bioassay data to attempt to understand the etiology of the disease, (2) the assumption that the bioassay serves to provide information about potential human carcinogens, and (3) the quantitative application of the results to the human population.

and using all possible precautions are still limited by the fact that humans are not genetically homogeneous 70-kg rats (or whatever test species was employed).

Gori (1980), in an article reviewing the biases inherent to the current mode of testing, concluded that

> In general, one can only (surmise) that current guidelines for the testing of carcinogens frequently introduce deliberate bias in order to enhance the probability of a positive response and that they ignore a number of sources of variability that cannot be controlled or are difficult to control with the available technology. Under current testing a carcinogen may go undetected in a particular assay, but just as likely a positive result may be valid for the particular species and test conditions utilized; current science cannot predict or explain the outcome.

It has also been observed that some "carcinogens" have demonstrated the capacity to increase the incidence of certain types of tumors while simultaneously causing a reduction in the incidence of other tumors. This observation does not appear to be due to spurious associations attributable to unrelated toxicity or to the early growth of lethal cancers censoring the appearance of those which appear only after a longer time (Weinberg and Storer, 1985).

These conflicting observations have been attributed to a number of confounding variables. An abbreviated list of these factors is found in Table 1. The diversity of these variables supports Gori's position (1980) and indicates that the determination of carcinogenicity cannot be separated from the experimental context---hence, the operational definition (Cumming, 1985). The recognition of context-dependent outcome also indicates that some carcinogens may not be carcinogenic per se but may provide for a conducive host environment for other causative factors present in the host, its environment, or its diet (Kolbye, 1976).

Even if all of these factors can be controlled, there are major pharmacokinetic differences in the ways different species absorb, metabolize, distribute, and excrete substances (Gillette, 1985). Also, most chemical carcinogens have multiple physiologic and/or toxicologic effects which could influence the outcome of a carcinogenicity bioassay. One is forced to conclude that the more a given substance is tested in different species, using an assortment of experimental protocols, the greater the probability of finding a positive carcinogenic effect in some test species. Similarly, while the types of confounding variables are different for the short-term tests, it can be assumed that the more a compound is tested the more likely a positive effect will be identified.

Table 1. Abbreviated list of factors known to influence the outcome of the chronic bioassay

1. Co-administration of agents which can affect drug metabolism or effect the appearance of the phenotype.
2. Contaminants of the food and water supply.
3. Alterations of the hormonal balance.
4. Environmental stressors (e.g., temperature control, increasing the number of animals in a cage, lighting, air flow).
5. Number of animals in the bioassay.
6. The route and vehicle of administration.
7. Sex, species, strain.
8. The pathological criteria used to evaluate the tumors.
9. The spontaneous background rate of the controls (the incidence of disease in untreated controls has been shown to vary as a function of laboratory and breeder).
10. Dose levels and dose rate in bioassay.
11. Immune status of animals.
12. Viral interactions.
13. Physiologic consequences of inbreeding.
14. Intercurrent disease and competing causes of death.
15. The statistical model used to evaluate the data.
16. Age of the test organisms at onset of exposure.
17. Number of initiated cells in the population under investigation.

Source: Gori, 1980; Yuspa and Harris, 1982; Crouch, 1983; Jones et al., 1983; Squire, 1984; Cumming, 1985.

2.1.3 Conclusions Regarding the Application of the Toxicologic Data for Hazard Assessment

The inference that the results of toxicologic testing are applicable to human health is implicit in biomedical and toxicologic investigations. This reasoning is logically extended to the results obtained from the long- and short-term bioassays for carcinogens and mutagens (NRC, 1983). These test systems have been used to generate an enormous amount of data on a variety of chemicals.

However, it has become apparent that as a chemical undergoes continued testing, using different test systems and protocols, apparent contradictions in the overall data can be produced. These qualitative and quantitative variations may be attributable to the observation that the results are only a reflection of the given assay with a particular chemical and thereby place restrictions on the interpretation and application of the observed effect (Gori, 1980; Cumming, 1985; Gillette, 1985; SGOMSEC, 1985). In an attempt to address these obstacles, the Interdisciplinary Panel on Cancer (1984) stated that

> Even though there is no basis for the exact extrapolation of risk from experimental studies to man, current advances, if exploited to the fullest, can provide a basis for distinguishing the degrees of risk from different carcinogens (authors' emphasis). The scientific characterization... requires scientific impartiality to review all appropriate data.

Given the conceptual and biologic distance between the test species and the human condition, and the vagaries of the bioassays, it is apparent that no generic procedure can be prescribed for human hazard evaluation using toxicologic data (Jones, 1983). For regulatory purposes, the immediate value of toxicologic evaluations is the capacity to arouse suspicion that a compound might be a carcinogen in humans. However, the application of the toxicologic data must also incorporate an implicit acknowledgment that biological models are extremely limited in their capacity to unequivocally identify human carcinogens, let alone be used to quantitate the risk to the heterozygotic human population. The potential for the inappropriate use of the test data will become even more critical as investigators continue to enhance the sensitivity of the test systems.

2.1.4 Classification of Carcinogens Using Toxicologic Data

As is now clear, major efforts have been dedicated to the study of potential carcinogens using a variety of laboratory techniques. Unfortunately, just as the notion of a strict definition of cancer is illusive, so is the definition of what represents a carcinogen. Frequently the toxicologic data are ambiguous (i.e., positive and negative effects in different test species or in the same species as the result of different protocols). Little is known about how a carcinogen or mutagen acts to produce its respective pathology or about the variety of external factors (e.g., stress, nutrition) which may contribute to the expression of the phenotype. As a result, unequivocal decisions are rarely made about the hazard represented by a given compound.

Given the paucity of information, it can be foreseen that the classification (and accuracy of the designation) of a compound as a carcinogen is dependent on the level of uncertainty that reviewers are willing to accept. These ambiguities have produced "schisms" in the scientific community, such that decisions about compounds---particularly those of

economic significance---are seldom accepted without significant controversy (see, for example, Lave, 1982). As Kolbye (1976) has observed,

> . . the emotional attitudes projected by various advocates sometimes approximate the emotional tenor generated by religious fanatics who seek to convert the world to their respective viewpoints. Many of these viewpoints are diametrically opposed to one another, and occur with almost infinite variation. Further, almost all are over simplifications of our knowledge and ignore our ignorance.

In turn, the current "operational definition" does not refer to a causal relationship but rather to a consensus among those reviewing the data (Kuhn, 1970). In other words, the decisive features in the classification process are not dependent upon particular results or a given approach but upon the amount of confidence the reviewers have in the studies which have generated the data under evaluation (Lave, 1982). This lack of a widely accepted methodology for the classification process results in a process wholly dependent on the nature of the criteria adopted for accepting the evidence of carcinogenicity (Saffiotti, 1977). Understandably, there is great variation in the estimates of the number of "known carcinogens." For example, a review published by the IRLG (1979) states that approximately 7000 chemicals have undergone long-term bioassay and that from this group 800-1000 demonstrated evidence of carcinogenicity; Clayson (1978) claims that there are 1000-1500 known carcinogens in test species; and IARC reviewed 585 chemicals, 304 of which were deemed to have demonstrated some evidence of carcinogenicity in test species (IARC, 1982).

2.1.5 Epidemiologic Methods for the Assessment of Chemical Carcinogens

In comparison with the toxicological test systems, epidemiology is best characterized as a discipline in which attempts are made to study a species (humans) in "free-living" situations. Of the scientific methods used to assess the carcinogenic risk associated with a physicochemical compound, the epidemiologic investigations are recognized as the "final" and most critical component of the health effects evaluation. The field is generally recognized as having two broad technical categories of data collection:

1. Descriptive epidemiology---includes summaries of self-reported symptoms, case reports, and ecological studies. In brief, these studies are utilized to generate hypotheses and explore potential relationships.

2. Analytic epidemiology---refers to the case control and cohort study designs. In brief, the analytic studies are used to test hypotheses, and, if possible, quantify a measure of risk (Interdisciplinary Panel on Cancer, 1984; OSTP, 1985).

The theories underlying the techniques employed by epidemiologists were originally developed for and have been shown to be very effective in the study of infectious agents. These exposures produce acute effects and have an "obvious" causal agent. With some modifications, the same types of techniques are now being applied to the study of low-dose exposures to chemical and physical agents which, in comparison to infectious diseases, produce effects that are generally delayed and of insidious onset. Low-level physicochemical exposures also generally produce more subtle effects (measured by the proportion of the population responding to an exposure) and have a longer period between exposure and the

manifestation of disease (latency). These issues make it very difficult to correlate an etiologic agent with a chronic disease, and, in most cases, all but eliminate the ability to evaluate the influence of modifying factors (OSTP, 1985).

Another problem with epidemiologic investigations of low-level exposures, which are the nature of most epidemiologic studies of cancer, is that development of standardized study protocols is impractical. In any study of humans, who exist in the environment of their choice, it must be recognized that each study is unique and must contend with a variety of variables beyond the control of the investigator. These factors include the extent and duration of the multiple exposures, the size of the exposed population, and the disease rate in the unexposed population. As a result, all studies have inherent constraints, particularly when an attempt is made to demonstrate a causal relationship to a physicochemical agent (IRLG, 1979; Doll, 1985; OSTP, 1985).

In summary, the significance of the epidemiologic study is that information is provided about human exposures and responses. However, the capacity to draw appropriate inferences from epidemiologic data, as with any scientific inquiry, is ultimately limited by the ability of the investigator to incorporate the appropriate variables. In this context, epidemiologic studies are distinguished from laboratory-based methods in that the epidemiologic assessment does not lend itself to a standardized protocol which can be implemented by any investigator to study any agent; an analogue of the protocols which are used to guide the toxicologic bioassays.

2.1.5.1 Assumptions and confounding issues associated with the epidemiologic investigations

Epidemiologic studies address the relevant population-at-risk; the studies are extremely informative when the results are unambiguous, but the data are rarely so clear. Both the strengths and weaknesses of the current epidemiologic techniques derive from the fact that the discipline is only partially an experimental science---it cannot control for the influence of the myriad of influencing factors. Yet, it does characterize events in the natural setting for humans.

In this context, the exposure assessment takes on the possibility for inadvertent distortion of heroic proportion. The distortion results from the fact that the "real world" (e.g., humans or other species in "free living" conditions) is extraordinarily complex; isolated, controlled exposures simply do not exist. The complexity is exemplified by the fact that chronic human disease is usually attributed to multiple risk factors. In turn, this leads to further uncertainties in the exposure and effects assessments (Brown, 1985).

Another complex issue in the interpretation of epidemiologic study results is how to regard the absence of statistical significance. By consensus, it has been agreed that negative data should not indicate the lack of risk (e.g., noncarcinogenicity) because the analysis could have been influenced by a wide variety of factors, including the aforementioned confounding variables, the sample size, or other fundamental problems. In regard to this issue, the Interdisciplinary Panel on Cancer (1984) stated that

> In epidemiology, as with other disciplines, it is impossible to prove a negative . . . however, negative results can be used to . . . indicate the limits within which a specific type of exposure will not affect the incidence of cancer in man.

In summary, the study of different human populations-at-risk is complicated by the multifactorial model of disease (Weiss and Liff, 1983), the limitations of exposure and effects assessments (Brown, 1985), and variations in the nature of the confounding variables (Sackett, 1979). These problems weave through the epidemiologic literature in the form of vast numbers of studies that can only be used for "hypothesis generation." In all likelihood, this can probably be attributed to the demands that quantitative studies put on the investigator; the technical aspects are difficult to perform, the results and analysis are complicated, the investment is great, and the overall interpretation is difficult (Paddle, 1980; Doll, 1985). Nonetheless, it must be emphasized that the (scientific and political) issues are so complex that simplification is bound to be of minimal value (Leviton, 1984). This point is stressed because the analytic studies remain the sole means of generating direct and quantitative estimates of increased human risk.

2.1.5.2 Conclusions regarding the epidemiologic data

Obviously, the difficulties of performing the analytic epidemiologic studies place limits on the number of compounds that can be studied using this study design. For example, of 585 chemicals, groups of chemicals, industrial processes, and occupational exposures evaluated by IARC, 541 lacked the epidemiologic data necessary for an evaluation of human cancer risk (IARC, 1982). The reasons for this are the consequence of the observational characteristics of the study designs and the inherent difficulties of this type of analysis. These difficulties include too short a period of exposure to make an epidemiologic study worthwhile; difficulty in identifying a large enough study population that has well-characterized exposure to the suspect agent without concurrent exposure to other compounds which could confound the analysis; lack of access to pertinent information; and, finally, a limited number of epidemiologists that are willing to make the commitment necessary to engage in these types of studies (Saracci, 1981).

Thus, as with results from the long-term bioassays, the assessment of carcinogenic risk using epidemiologic data must be tempered by the judgment and experience of qualified professionals. Although the purpose of the analytic study design is to estimate the magnitude of the measured effect (i.e., increased risk for disease), the interpretation has to include insight into a wide variety of other considerations. This would include, for example, information obtained from toxicologic test systems. Data from the latter can be used to demonstrate the plausibility of the agent-disease relationship and elucidate the biologic mechanisms involved in the development of the particular pathology (IRLG, 1979; Interdisciplinary Panel on Cancer, 1984; Doll, 1985).

2.1.6 Classification of Carcinogens Using Epidemiologic Data

Ironically, the characteristic which distinguishes the epidemiologic investigation and offers such potential relevance to human health risk assessment is also that which imposes caveats on the interpretation of the data---i.e., the study of humans in the environment of their choice. This factor introduces a variety of confounding variables which can influence the results and interpretation of a study (Sackett, 1979).

The uncertainties in interpretation of the data place significant restrictions on the use of epidemiologic data to classify the carcinogenicity of chemicals (Doll, 1985; OSTP, 1985). Consequently, it is recommended that (1) single studies not serve as the consummate test of hypotheses, (2) each study be evaluated individually for robustness and weight, and (3) the

studies be supplemented with information obtained on laboratory test systems. These issues are so demanding that widely accepted guidelines have not yet been developed for standardization of the details of the design or analysis (NRC, 1983; Interdisciplinary Panel on Cancer, 1984). As with the long-term bioassay, this lack of consensus is reflected in the number of "known human carcinogens", which varies as a function of the criteria employed to assess the data. For example, within a one-year span of time, two agencies with access to essentially the same information developed estimates which ranged from 23 agents (IARC, 1982) to as many as 88 potential agents (U.S. Department of Health and Human Services, 1981).

2.2 METHODS FOR THE USE OF TOXICOLOGIC DATA FOR HAZARD ASSESSMENT

Much effort has been expended in search of the best method for the evaluation of human toxicity from exposure to carcinogens. Of the methods currently used, only analytic epidemiology offers direct evidence of hazard to humans, let alone the capacity to quantify the risk. However, these studies are difficult and expensive. As such, relatively few chemicals have been evaluated using this study design.

In contrast, enormous amounts of information have been generated in the wide variety of toxicological short-term and chronic bioassays. Each of these methods offers its own inherent strengths and limitations and, when utilized properly, can provide useful information. Lacking objective criteria for the use of toxicologic data for the quantitative estimate of human hazard or risk, the critical issues reduce to the question of how to most effectively integrate the data into a meaningful decision-making process.

2.2.1 Correlation of Tumorigenicity and Mutagenicity

At this time, none of the toxicologic test systems has clearly been shown to be predictive of human cancer. This is consistent with the fact that the mechanisms, causes, and etiology of the disease remain poorly defined phenomena. Hence, one cannot expect to develop biological models to provide the kinds of information that will be available when the etiology of cancer is better characterized (Reichsman and Calabrese, 1978).

Nonetheless, in the near term, data from laboratory investigations are likely to continue to be the sole source of information on the hazard posed by many environmental, occupational, and dietary contaminants. Therefore, it is imperative to develop methods which would allow for the scientifically defensible setting of priorities to guide research needs (Doll and Peto, 1981; Walsh et al., 1982). This is particularly true for the short-term tests, given their inherent potential for rapid and comparatively inexpensive results.

Several investigators have attempted to test, and thereby evaluate, the predictive properties of the bioassays used to measure mutagenicity (Meselson and Russell, 1977; Bartsch et al., 1980; Bartsch et al., 1982; Bartsch and Tomatis, 1983; Tennant et al., 1987; Piegorsch and Hoel, 1988). The motivation for these investigations is a theorized similarity between the basic biological processes responsible for mutations in short-term test systems and the tumors observed in the chronic bioassay. However, so little is understood about the nature and mechanisms leading to tumorigenicity and mutagenicity that it is difficult to use this type of correlation as a means of further understanding the observed pathologies.

Only a limited number of the short-term test systems currently used for mutagenicity analysis have actually been evaluated in terms of their potential predictive value. In general,

the correlations have been limited to the activity of carcinogens (classified using toxicologic and/or epidemiologic data) in different strains of *Salmonella typhimurium* (Brusick, 1983). Although these analyses have been used to defend the use of the short-term test systems, there are reasons to suspect the approach has been implemented inappropriately. The following section reviews some of the major issues influencing the analysis of these types of correlations.

2.2.1.1 Qualitative correlations between carcinogenic Activity and the activity of chemicals in the short-term tests

A method that has frequently been used to attempt to validate short-term tests is to correlate the qualitative results (positive or negative) of the long- and short-term bioassays. The appraisal is based on the assumption that the short-term data represents a "screen" for the long-term bioassay (Cooper et al., 1979). Data used in the evaluation are analyzed using methods developed in the medical and epidemiologic literature (Lilienfeld and Lilienfeld, 1980). To understand the analysis, it is important to first discuss the origins of the technique, then the application to the toxicologic data.

Epidemiologic Screens

The epidemiologic notion of screening derives from the application of a simple test or procedures which can rapidly and accurately ". . . sort out apparently well persons who have a disease from those that do not. A screening test is not meant to be diagnostic" (Last, 1983). In other words, the approach is not designed to offer a definitive appraisal--it is only used to obtain descriptive information about a population.

Epidemiologic screening tests are evaluated by quantitative assessments of their (1) validity (sensitivity and specificity) and (2) reproducibility or precision (Lilienfeld and Lilienfeld, 1980). To develop an epidemiologic screen, a rigorously characterized population (with and without a given disease) is tested using a technique which allows for a rapid disease evaluation. The results are then compared to the more definitive original diagnosis.

To select a screening tool, valid and reproducible tests are selected for population-based surveys. This allows for the calculation of the positive predictive values of the different tests. This value is a function of both the screening tool and the prevalence of "true positives" in the test population.

Toxicologic Screens

As noted earlier, when using the toxicologic data, compounds are operationally defined as being carcinogens or mutagens based on the results of chronic *in vivo* or short-term tests, respectively. The comparative simplicity and low cost of the mutagenicity assays have placed the mutagenicity bioassays in a pivotal role in terms of identifying potential carcinogens. However, the significance of the results obtained using the short-term tests still needs to be resolved (Doll and Peto, 1981; OSTP, 198_).

To assess the utility of the short-term tests as "screens" for potential carcinogens, an analysis has been developed which is similar to the technique used to evaluate epidemiologic screens. The approach is based on correlations between compounds identified as carcinogens and mutagens, and noncarcinogens and nonmutagens. Thus, in theory, a short-term test system is "validated" (or demonstrated to be "reliable") by correctly distinguishing compounds

that are, or are not, classified as human or animal carcinogens (Cooper et al., 1979; Tomatis et al., 1982; Brusick, 1983). However, this selection is recognized as being biased in that few chemicals have demonstrated adequate evidence of a lack of carcinogenic activity (IARC, 1982; Tomatis et al., 1982).

An example of the technique used in a toxicologic screening analysis is as follows:

Outcome in short-term test	Animal or human carcinogen		
	YES	NO	TOTAL
Positive	a	b	a + b
Negative	c	d	c + d
Total	a + c	b + d	N = a + b + c + d

Where: a= mutagenic carcinogens
b= mutagenic noncarcinogens
c= nonmutagenic carcinogens
d= nonmutagenic noncarcinogens

This procedure allows for the calculation of summary measures similar to those for the evaluation of the epidemiologic screen. These measures, i.e., sensitivity (mutagenic carcinogens/total carcinogens; a / a + c), specificity (nonmutagenic noncarcinogens/total noncarcinogens; d / b + d), and positive predictive value (mutagenic carcinogens/ mutagenic carcinogens + mutagenic noncarcinogens; a / a + b) are then used to characterize the "validity" of a test system (Cooper et al., 1979; Tomatis et al., 1982; Brusick, 1983). Using this type of an approach, investigators have claimed to have demonstrated correlations between the *in vitro* and *in vivo* data ranging from 50% - 95% (Brusick, 1983).

The fallacy of this reasoning is that unlike the epidemiologic screens there are no well defined, rigorously determined standards to use for the evaluation of the toxicologic screen. The lack of a scientifically valid standardized system for the evaluation of bioassay data results from the general lack of knowledge of: (1) the genesis and development of the disease, and (2) the chemical-context dependent nature of every bioassay. Evaluation of the toxicological screens is therefore essentially limited to determining whether or not, and to what extent a given bioassay followed a prescribed protocol. Consequently, if a compound has been tested in a variety of bioassays its classification as a carcinogen/noncarcinogen or mutagen/nonmutagen is dependent upon the evidence the reviewer is willing to accept. Also, given that few chemicals have been adequately shown to be noncarcinogens, it is inevitable that the test sample will contain a high proportion of mutagenic carcinogens. This can force the predictive value to appear to be higher than when the proportion of carcinogens is lowered (Purchase, 1980; Tomatis et al., 1982; Brusick, 1983).

Even if classification systems and protocols were standardized, it is possible that two strains of rodents (same species and sex) might respond differently to the same chemical. In turn, the mutagenicity data could appear to be "predictive" for one, but "false" for the other. These apparent contradictions exist as the result of inadequacies of our understanding of both the "short-term" test systems used for the prediction, as well as the "long-term" bioassay used as the standard (Brusick, 1983; Clive, 1985; Madle et al., 1986). Concluding, the limitations

of the "screening" approach to validate the mutagenicity data are consistent with (1) the uncertainties regarding the nature of the disease, and (2) the lack of theory to assist in the interpretation of the multiple results generated by the test systems (NRC, 1984). Since many factors have been shown to influence the outcome of the bioassays, the classification of a compound as a noncarcinogen or nonmutagen must be recognized as being tentative and subject to reclassification (Clive, 1985). This can occur if different criteria are used to assess the existing data or if the compound is evaluated with subsequent bioassays using different protocols.

This tentativeness is also true for the classification of a compound as a human carcinogen. Given the number of variables which can influence the epidemiologic estimates of risk, comparatively few compounds have been definitively categorized as human carcinogens or noncarcinogens (Saracci, 1981; OSTP, 1985). Viewed from this perspective, it is difficult to justify using a "screening" approach to validate the mutagenic test systems, regardless of whether test animal or human data are used as the standards. Further, since the analysis is limited to the use of qualitative information (positive or negative), the approach cannot distinguish the severity of the hazard represented by different compounds, nor provide the detailed type of information best suited for human hazard assessment (Squire, 1984).

2.2.1.2 Quantitative correlations between carcinogenic activity and the activity of chemicals in the short-term tests

Attempts have also been made to develop a quantitative correlation between *in vitro* and *in vivo* data (Tomatis et al., 1982; Bartsch, 1983). The implication of such a correlation is that it is possible to detect potent carcinogens as the result of a strong positive response in mutagenicity test systems. These types of correlations would obviously be very useful for the estimation of hazard represented by chemicals and complex mixtures in the absence of sufficient carcinogenicity data.

Quantitative correlations between tumorigenicity and mutagenicity are much more difficult to evaluate than the qualitative correlations. Only a few quantitative correlations have been published, but these have not produced results which demonstrated sufficient reliability to gain general acceptance of the approach (Meselson and Russell, 1977; Ashby and Styles, 1978; Casto, 1981; Tomatis et al., 1982; Barr, 1985). Reservations about this type of correlation can be summarized as follows: (1) variables governing the tumorigenic response cannot be reproduced *in vitro* (Ashby and Styles, 1978; Rinkus and Legator, 1979; Tomatis et al., 1982), (2) no method of expressing carcinogenic and/or mutagenic potency has been widely accepted (Tomatis et al., 1982; Barr, 1985), and (3) the activity of a compound in a given bioassay (*in vitro* and *in vivo*) has been demonstrated to be dependent on the protocol used for the experiment (Ashby and Styles, 1978; Casto, 1981; Barr, 1985). These issues obviously make quantitative comparisons very difficult.

2.2.2 Use Of Toxicologic Data For Regulatory Purposes

An obvious method that could be used to assist in the setting of priorities is the calculation of carcinogenic potencies. However, as noted, compounds tend to be characterized as carcinogens using an operational definition. This is experimentally unavoidable because of the apparent independence of applied dose and severity of effect and a lack of understanding of the underlying mechanisms.

Further, there are no data to indicate that humans would demonstrate even a remotely similar susceptibility as the experimental species used for the experiments. This is unlikely because (in addition to the previously detailed limitations):

1. The test animals represent a highly inbred group (whose breeding has focused upon ensuring a high spontaneous rate of cancer and a high, uniform, susceptibility to insult) exposed to controlled test conditions and whose response can change using different conditions (e.g. changing the route of administration, alterations in the dose-rate, etc.) (Mayneord and Clarke, 1978; Cumming, 1985; Jones, 1985; SGOMSEC, 1985),

2. Responses may be unique to a particular strain; it may not even be quantitatively predictive of other strains of the same species-- even for direct acting agents (Hegsted, 1975; Rice and Perantoni, 1983) and,

3. The human species is composed of a genetically heterogeneous population, exposed to a variety of uncontrolled conditions; both factors are known to influence susceptibility to xenobiotics (OSTP, 1985; SGOMSEC, 1985; Vesell, 1985).

The consequence of these issues is that no single method has been developed (and widely accepted) to quantify the potency of carcinogens to test species, let alone humans (Barr, 1985). To compensate, and offer regulators guidance, three general approaches have evolved: (1) "mechanistic classifications" based on *in vivo* data, (2) "the weight of the evidence", and (3) "quantitative risk assessment."

2.2.2.1 Mechanistic classifications

A large body of research suggests that cancers proceed through a number of stages which can be differentially affected by different chemicals. In general, these stages are recognized as having distinct characteristics. The stages are broadly classified to include the pathologic states of initiation and promotion. Additionally, some investigators have suggested that initiation and promotion are comprised of substages (OSTP, 1985).

Chemically induced initiation appears to result from the covalent binding of the compound (or its metabolite) to DNA and other cellular macromolecules. It is posited that this covalent binding is associated with the production of a mutation in the exposed cell. Current theory also holds that if the initiated cell undergoes division without repair the damage becomes irreversible (Yuspa and Harris, 1982; Weisburger and Williams, 1986). Many investigators believe that this effect is modeled by mutation in the *in vitro* test systems (OSTP, 1985; Weisburger and Williams, 1986).

The molecular events associated with tumor promotion are much more obscure than those producing initiation. Cells which have undergone the stages leading to the neoplastic conversion are thought to remain dormant or suppressed by tissue homeostatic factors. A promoting stimulus is believed to facilitate the loss of the homeostatic control mechanisms leading to the deregulation of the initiated cell. It is hypothesized that this ultimately yields the undifferentiated cell type characterized as a malignant transformation (Yuspa and Harris, 1982; Weisburger and Williams, 1986). The theorized attributes of initiating and promoting agents, deduced from observations of *in vitro* bioassays, are summarized in Table 2.

Table 2. Hypothesized properties of initiating and promoting agents

Hypothesized properties of initiating agents
1. Carcinogenic by themselves
2. Must be given before promoting agents
3. A single exposure, with no apparent threshold, is sufficient to produce the effect
4. Action is cumulative and irreversible
5. Mechanism of action appears to be related to electrophiles (most require metabolic conversion to produce the electrophiles) that covalently bind to macromolecules
6. Either the parent molecule or its metabolite produces a mutation
Hypothesized properties of promoting agents
1. Not carcinogenic without initiation
2. Must be given subsequent to initiating agent
3. Induced pathology may be reversible
4. Individual exposures do not appear to produce cumulative effects (i.e., effects require prolonged and repeated exposures)
5. Metabolism and covalent binding not required for effect
6. Not necessarily mutagenic
7. Effect appears to have a threshold dose

Source: Yuspa and Harris, 1982; Weinstein, 1985; Weisburger and Williams, 1986.

These observations have allowed for the proposal of a mechanistic classification scheme (Kroes, 1983; Weisburger and Williams, 1986). According to these authors, although current knowledge does not allow for a rigid classification of carcinogens, two broad categories can be distinguished: (1) genotoxic--referring to agents which can interact with nucleic acids directly, and (2) epigenetic- a catch-all phrase for all other mechanisms of action. The latter is subdivided into a variety of classes including solid-state carcinogens, hormones, immunosuppressors, co-carcinogens, and promoters.

The capacity to differentiate carcinogens in this way has lead some investigators to suggest that only those agents which theoretically have the capacity to act as an electrophile *in vitro* should be recognized as "true carcinogens." In contrast, compounds which can induce transformation without direct chemical interaction with the genome (e.g., their action is mediated via secondary mechanisms or they act to modify the activity of another endogenous or exogenous agent) would not be defined as carcinogens (Clayson, 1978; Kroes, 1979).[14]

This type of classification scheme leads to arguments directed at how the risk of different categories of chemicals should be prioritized and regulated. Theoretically, those acting by epigenetic mechanisms may demonstrate a threshold dose, whereas those interacting directly with the genome may not be safe at any level of exposure (Kroes, 1979; Kroes, 1983; Rodricks and Taylor, 1983; Weisburger and Williams, 1986). This has led some investigators to suggest that only initiators should be regulated as carcinogens (Clayson, 1978; Kroes, 1979). Despite the apparent logic of this classification scheme, there are a variety of issues which confound its utility for regulatory activities. These include, for example:

1. It cannot be determined if all of the tumors observed in a given organism are produced by the same mechanism. Certain carcinogens appear to act as initiators for some types of tumors and promoters for other types of tumors (Day and Brown, 1980; Peto, 1982).

2. The mechanism by which a compound can elicit a carcinogenic response can change as a function of dose. Thus, characterization of potential mechanisms becomes blurred at the very high doses routinely employed in the long-term bioassay, in contrast to the comparatively low dose human exposure (Rodricks and Taylor, 1983; Williams and Weisburger, 1986; Ames et al., 1987). For example, cyclophosphamide has been demonstrated to act as an immunosuppressant at "low" doses and a genotoxin at "high" doses (Williams and Weisburger, 1986).

3. Some non-genotoxic agents appear to be acting via oxidation-peroxidation pathways to actually induce DNA damage via a secondary mechanism (Ames et al., 1987). This may account for the fact that continuous chronic exposures to these agents in experimental settings are necessary for the development of neoplasia

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[14]Obviously, the classification of a compound as a carcinogen is of utility only if the compound increases cancer in man, who lives in a contaminated world. In contrast, the compound which increases the incidence of cancer in the barrier nursed test species is most appropriately recognized as (1) providing information of significance in the determination of potential hazard and (2) in understanding the "basic science" of the mechanisms leading to the production of malignant transformation.

(Flavin, 1984; Weisburger and Williams, 1984). The wide variations in human exposures indicate that these observations may not be reflective of human exposure and response scenarios.

4. Some carcinogens (e.g. asbestos and diethylstilbestrol) have demonstrated the capacity to induce chromosomal aberrations, yet they were originally classified as epigenetic agents on the basis of *in vitro* bioassays (Barrett et al., 1983).

5. Both genotoxic and epigenetic agents can ultimately produce the effects that Saffiotti (1977) claimed were the characteristics of chemicals that can produce self-replicating toxic effects. Yet, the latter group, may be able to produce a reversible lesion under certain conditions and demonstrate a dose-dependent risk for the expression of its carcinogenic potential (Stott et al., 1981). Nonetheless, both are capable of producing a malignant transformation.

6. Certain compounds are necessary for normal metabolism, yet at high doses have been demonstrated to be carcinogenic. For example, at elevated doses certain steroids (hormones and vitamins) and trace elements can produce a frank neoplasia (Williams and Weisburger, 1986).

7. The assay systems that tend to be used to make these types of designations may not detect all types of chemical-DNA interactions. Nor can they accommodate the biologic events that may occur at the level of the intact tissue which may influence the capacity of a compound to be carcinogenic (OSTP, 1985).

8. It has been suggested that all mammalian organisms are either born with or, given the number of initiating agents in our environment, invariably acquire initiated cells. If this is operationally correct, in the "natural environment", promotional stimuli could be acting as the rate limiting steps in the development of cancer (Slaga, 1980; Jones, 1983). This possibility obviously challenges the notion that initiators (as a class) should be more rigidly regulated than promoters (as a class).

Thus, a chemical may be broadly characterized as a carcinogen because it is a potent initiator, potent promotor, or some combination of both (Kroes, 1983; Bernstein et al., 1985). If it is primarily an initiator, the chemical would theoretically have a low potency in a tumorigenesis bioassay unless an artifact of the experimental protocol acted as a promotional stimulus. These artifacts may be inherent in the animal strain or they may be externally related to substances or factors in the laboratory environment (Kroes, 1979; Jones et al., 1983).

Accordingly, if the chemical is primarily a promotor, the incidence of the tumorigenic response would be dictated by the presence of initiated cells at the site of the hyperplasia in concert with the magnitude of the exposure to the promotional stimulus (i.e., intensity and duration of the exposure). The "spontaneous" production of initiated cells may result from viruses, errors in replication, natural radiation, or toxic chemicals present in the food or laboratory environment (Jones et al., 1983; Weisburger and Williams, 1984).

In summary, the "mechanistic classification" scheme provides a useful means of organizing carcinogens into broad categories. However, the carcinogenic process may not

always proceed through identifiable, discrete stages (IARC, 1982)[15]. Nor is it always possible to use the experimental evidence to demarcate conclusive distinctions between "epigenetic" and "genotoxic" mechanisms of action (OSTP, 1985). Thus, it appears that there is not sufficient understanding of these events *in vitro* or *in vivo* to use such a classification scheme for guidance in regulatory activities (Rinkus and Legator, 1979; IARC, 1982; Squire, 1984; Weinstein, 1985).

2.2.2.2 Weight of the evidence

This approach has been adopted by the International Agency for Research on Cancer (IARC) and has been employed as a means of utilizing the available data, without endorsing experimentally unresolvable issues. In order to categorize the data without going beyond the limits of experimental verification, information is formally arranged into "degrees of available evidence." Data from epidemiologic and animal studies are categorized as having:

1. "Sufficient" evidence (group one)-- the chemical, group of chemicals, industrial processes or occupational exposures is deemed to be carcinogenic to animals and/or humans. This category is used to reflect sufficient evidence from toxicologic and/or epidemiologic studies to support a casual association between the agent and cancer.

2. "Limited" evidence (group two)-- the chemical, group of chemicals, industrial processes or occupational exposures is probably carcinogenic to test species and/or humans. The category is used to reflect data that is almost "sufficient."

3. "Inadequate" evidence (group three)-- the chemical, group of chemicals, industrial processes or occupational exposures cannot be classified as to its carcinogenicity to test species and/or humans.

Group two is further subdivided into "group A" (limited evidence in humans, but sufficient evidence in test species) and "group B" (inadequate evidence in humans, but sufficient evidence in test species) (Saracci, 1981; IARC, 1982). In their review of the toxicologic literature, the IARC has reviewed information on approximately 600 chemicals, and has identified more than 140 as having sufficient evidence of being a carcinogen in test species. The organization does not believe that a quantitative correlation can be developed at this time between the toxicologic data and risk in humans. However, they suggest that the toxicologic data offers a practical perspective and that these compounds should be treated as if they represented a carcinogenic risk to humans. Although the approach cannot distinguish degrees of potential hazard (i.e., all carcinogens are not equally "potent"), the IARC believes that, given the limitations of our understanding of the events leading to the expression of cancer, this is the most appropriate means of offering guidance to regulatory agencies (IARC, 1982).

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[15]It is important to recognize that the discrete stages are based upon observations of test species, exposed to single compounds, in highly controlled conditions. The natural history and progression of chemically induced disease may be quite different given the complexities of man's exposures.

2.2.2.3 "Quantitative Risk Assessment" (QRA)

In the absence of epidemiologic data, this method has been used as a means of calculating a population-based estimate of the magnitude of the health impact associated with exposure to a carcinogen. The rationale of the approach is the development of methods which will allow for the quantitative determination ". . . of the magnitude of the risks as a basis for setting priorities and balancing risks against social and economic factors" (Anderson et al., 1983). A variety of mathematical models, which are qualitatively and quantitatively different, have been proposed for this purpose (Altshuler, 1981; Brown and Kozial, 1983; Downs, 1985).

For example, in the evaluation of toxicologic data, the Environmental Protection Agency's Carcinogen Assessment Group (CAG) uses quantitative risk assessment as part of a two-step process. The first step is to identify potential human carcinogens using a "weight of the evidence" approach, similar to the IARC procedure. Following the qualitative evaluation, the second step involves the coupling of a preselected model (the CAG uses a "linearized multistage" model) with experimental data obtained from long-term bioassays (Anderson et al., 1983; Anderson, 1985).

The "linearized multistage model" was selected because the CAG committee believed that it reflected the "correct" and "most conservative" assumptions about chemically induced disease (i.e., no-threshold, irreversible changes, linear relationship between low doses and effects, multiple stages, etc). The experimental data is "analyzed" by forcing it to fit the model; data from the higher doses are serially removed from the dose-response relationship until an acceptable fit is obtained. The resulting slope, at low doses, is taken to be a dose-dependent index of potency ("unit risk") and expressed as the 95% upper-bound of the risk estimate. The results are interpreted as being representative of the lifetime risk of being diagnosed with cancer after exposure to a fixed daily dose of the compound for a fixed period of time (72 years) and in the absence of competing causes of death (Anderson et al., 1983).

The limitation of the "conservative assumptions" employed in this modelling process is that they are actually reflections of the current dogma and not capable of being addressed experimentally.[16] Also, because a wide variety of factors can influence the results obtained from the chronic bioassay, the data are most appropriately recognized as being semiquantitative and thereby possessing limited external validity. Hence, the use of statistical

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[16]The issue of using the "most conservative assumptions" is widely debated. It is frequently held that this is the most appropriate approach because the evaluation of risk deals with human health. However, as noted by the National Academy of Sciences (1983), a scientific assessment should neither be "conservative" or "liberal." These are sociopolitical policies, whose resolution should be mandated by legislative fiat.

To distinguish these activities, the NAS (1983) characterizes risk assessment as an impartial scientific evaluation of the data and, if possible, the characterization of the risk. Determinations of the scientific and social policy considerations are the purview of risk management. The assessment is the realm of the scientist; the management most appropriately left to the lawyer and regulator. It is extremely important that the scientific characterization of risk does not incorporate subtle biases by merging these activities (Barnard, 1984). In order to remain credible, particularly as a developing science, the explicit division of these two activities is of paramount significance.

models can be misleading and produce a false sense of confidence in the estimates of "human risk."

One manifestation of this is the tendency to misinterpret the results as representing a legitimate estimate of the probability for human risk of disease with exposure to the agent (see, for example, Marshall, 1982; Crouch and Wilson, 1987; Lave, 1987). This tendency derives from the ease with which one can become mesmerized by the ". . . strings of precise numbers being churned out by computers and forget that the biological data going in aren't anywhere near so precise" (Winrow, 1982). Consequently, the biases and limitations of the statistical model are being superimposed on the bioassay data as a means of characterizing an unknown, potentially unmeasurable, human response.

Despite the limitations, the use of the technique has grown because it allows for an apparent reduction in the complexity of the data; the presentation of complex results as single numbers is obviously very appealing to regulators. However, it must be emphasized that the basis of QRA is the application of a statistical model to data obtained from a single animal model (where a positive result is considered, a priori, to represent a human carcinogen). The result of this activity is a simple extrapolation employed to develop an estimate of "human risk," following an idealized lifetime exposure. Unfortunately, critical review of this technique leaves the impression that, at best, the QRA approach is providing a ". . . first estimate of incidence at low dose levels in the strain and species under consideration" (Purchase, 1985). It is quite a different problem to predict the response to a substance in a heterogeneous human population living in a heterogeneous environment (Gillette, 1985).

Concluding, the attempts at quantitative risk assessment must be recognized as pioneering efforts, which offer useful information for priority setting, but the limits of the toxicologic data overwhelm any useful quantitative product, per se. In brief, there is no theory to assist in the selection of the experimental data (assuming a number of experiments are available) to be used for the analysis. Nor, given the complexities of multistage, multifactorial processes which appear to be responsible for the manifestation of the pathology of cancer, are the statistical models informative about (1) the dose-response behavior at the low doses of environmental exposures, (2) the validity of the assumptions employed for the interspecies conversions, or (3) the multiple exposures which constitute the human experience. Hence, the QRA approach is powerful (and even seductive) in its capacity to generate "predictions" about human health, but almost totally devoid of quantitative experimental justification or comparison with practical experience (Purchase, 1985). As asserted by Peto (1981), the desire to summarize toxicologic data with existing statistical models as a means of estimating human risk is simply ". . . not scientifically respectable and never will be." However, advances in the understanding of the disease processes and the mechanism of action of carcinogens will undoubtedly produce better models.

2.3 RELATIVE POTENCY

The purpose of bioassay techniques (short-term and long-term) is to determine a quantitative relationship between a xenobiotic and the magnitude of the response it can elicit. The potency of a compound is then expressed as a function of dose-dependent effects. Traditionally (where the effect is not malignant transformation), this generally takes the form of a relationship between the severity of the induced pathology and the intensity of the exposure. If carcinogens could be classified as a function of their "potency," this type of

measure would obviously provide the most efficient means of appraising the hazard represented by the compounds capable of producing this reponse.

Unfortunately, the current understanding of the mechanism of action of carcinogens (i.e., the severity of the response, in the affected individual, is independent of dose) precludes the use of the classical definition of potency. A review of a variety of alternative suggestions to calculate carcinogenic potencies can be found in Barr (1985). As with the mathematical modeling procedures, these approaches tend to estimate potency as a function of the results obtained in single experiments.[17]

However, given the state of the science,[18] it can be argued that these types of potency estimates (for mutagenicity and tumorigenicity) are limited to the activity of a compound in a particular "setting" (e.g., particular protocol, test organism, etc.) (Cumming, 1985). As noted, this context dependence for the production of the effect restricts the definition of carcinogenic and mutagenic potency to an operational one. Therefore, past attempts to estimate potencies and/or validate the short-term bioassays are actually testing context-dependent relationships; i.e., the activity of a compound using one protocol vs. the activity of a compound using another protocol.[19]

Only a limited number of the variables responsible for these observations can be identified, and of these, only a small fraction can be attributed to inherent biological properties of the test systems. In turn, justification for the use of the short-term test systems clearly cannot be based on an understanding of the mechanisms responsible for the observed endpoints. Nor have any systematic empirical studies demonstrated that a given short-term test system is more or less predictive of carcinogenicity than any other.

Nonetheless, the results of the correlations between effects observed in long- and short-term bioassays tend to be employed to "validate" the use of short-term tests as a means of "screening" for potential carcinogens. These results have also been used to infer that the carcinogenic effect may be due to a very limited form of biologic damage (e.g., point mutation) (Cairns, 1981; Dunkel, 1983). However, evidence collected over the past three decades indicates that this is probably not valid (Brues, 1958; Cairns, 1981; Dunkel, 1983;

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[17]For example, some investigators have recommended the use of the TD50 (i.e., the chronic dose rate which produces tumors in 50% of the test animals by some given fixed age) as a means of characterizing the potency of a chemical (Peto et al., 1984; Sawyer et al., 1984). Using actuarial adjustments to compensate for intercurrent mortality, the group has published a data base with results from 3000 experiments on 770 substances (Gold et al., 1984). This data base is limited to studies which followed the NTP protocol.

[18]The "state of the science" refers to the limitations of the theory underlying the application of the results obtained from short-term and long-term tests. It is used to allude to restrictions imposed by (1) the lack of understanding of the factors which are responsible for the production of the effects, (2) the limitations of study designs and inherent biases of the bioassays, (3) the variations in the interpretation of the results, and (4) the assumptions and errors made during the calculation of the summary estimate of potency (Barr, 1985; Squire 1984; Gori, 1980; Ashby and Styles, 1978).

[19]These correlations can take many forms but in general are based on groupings by test systems (for example, correlations between strains of salmonella, rats vs. mice, different strains of rodents of the same species, rats vs. a strain of salmonella, etc.).

Yunis, 1983; Natajaran and Obe, 1986). Thus, a more appropriate measure of the potential correlation between long-term and short-term tests must take into account the possibility that (1) a compound may only produce a particular type of effect, (2) this effect may only be produced under certain conditions, and (3) a variety of molecular events may contribute to the progression of malignant cells (Bridges, 1980; Bridges et al., 1983; Barrett et al., 1983). Further, any correlation must be compatible with "operational definitions" of the observed effects.

To address these needs, correlations between short-term and long-term tests may be best approached using a technique which allows for an integration of endpoints. Rather than grouping the information by test systems (e.g., point mutation in Salmonella; liver tumors in mice), this integrative type of an approach would aggregate experimental data (e.g., "pool" mutagenicity data and "pool" tumorigenicity data). While this technique is not designed to address mechanistic hypotheses, it could provide the means for a less biased assessment of the utility of the short-term test systems.

Concluding, the short-term tests are obviously of great significance in terms of their applications to the study of the mechanisms of chemically induced genetic damage as well as in terms of their potential role in the identification of carcinogens. The latter function is currently limited by the quantitative and qualitative variations in the results obtained from different experiments. However, lacking justification to suspect otherwise, it must be acknowledged that all chemical-context interactions produce information of some utility to hazard assessment. Hence, rather than comparing specific bioassays, it is probably apropos to use as much information as possible to test the correlation between the bioassays for mutagenicity and the bioassays for tumorigenicity. A relative-potency approach can provide the framework that would be necessary for these types of correlations.

2.3.1 Relative-Potency Approach

The relative-potency approach has been used widely in radiation biology and classical pharmacology as a means of comparing the effectiveness of differenct types of agents (e.g., radiation and pharmacologic, respectively) to produce a given effect. Conceptually, this comparison is facilitated by the transformation of data, obtained from a variety of bioassays, to a common scale (Casarett, 1968; Goldstein et al., 1974). The relative potency of an agent is calculated by comparing the amount of an agent (chemical or radiation) necessary to produce a given effect, relative to another agent, not by comparing the effect produced by equal amounts of different agents (Casarett, 1968). A generalized form of relative potency can be expressed as:

$$\text{Relative Potency} = \frac{\text{Dose of a reference agent needed to produce an effect of a given magnitude in a particular bioassay}}{\text{Dose of another agent needed to produce the same magnitude of the same effect in the same bioassay}}$$

Since the toxicologic bioassays have been demonstrated to vary in response to many endogenous and exogenous factors, ideally, the relative potency calculation would include extensive information on the bioassay used in the comparison (Goldstein, 1974). For example, to compare long-term bioassays, relevant factors which could affect the outcome of the experiments could include: all experimental animals being purchased from the same breeder at the same time, all being of the same sex, species and strain, maintained on the

same diets, having similar hormonal status, all exposed via the same route of administration, and all experiments being done in the same laboratory. However, pragmatically it is apparent that it will not be possible to compensate or standardize for all of the variables which could affect the "context-dependence" of the bioassays. Ergo, investigators are limited to incorporating as many variables as they have control over.

In summary, regardless of the area of study, it is evident that no measure is an "absolute" in and of itself. It is likely that this self-limiting nature of individual measures is responsible for the broad applications of "relative measures." The utility of these approaches derives from the provision of a framework for the direct comparison of data through the expression of results as being "relative" to a "reference." Thus, the conceptual tool offered by this approach derives from the capacity to express data on an ad hoc--but common scale. This facilitates the comparison and evaluation of diverse data sets. Although not usually explicitly stated, these conventions are implicit in the current applications of "quantitative risk assessment" procedures using toxicologic data (Anderson et al., 1983; Anderson et al., 1985).[20]

2.3.1.1 Examples of historical applications of the relative potency approach

Different types of radiations have been demonstrated to have very different types of physical properties and capacities to induce biological damage. In order to standardize these observations and thereby be able to "directly" compare different types of radiation-induced damage, radiation biologists use a relative potency approach. The application of the relative potency approach in experimental radiation biology is called the relative biological effectiveness (RBE).

An RBE is defined as the ratio of the absorbed dose from one type of radiation to the absorbed dose of a reference radiation, where both are required to produce the same degree of biological effect under the same conditions. Generally, the reference radiations have been limited to ^{60}Co gamma rays or 200 - 250 Kev X-rays (units of dose are expressed in rads). This allows for radiation-induced damage, observed in vastly different experimental situations, to be expressed as a function of an equivalent reference dose (Casarett, 1968; Hobbs and McClellan, 1986).

Pharmacologic applications are very similar to the approach used by the radiation biologists. For example, the approach is frequently applied to circumstances where an investigator wishes to evaluate the capacity of a "test compound" to produce a given effect. In this context, the potency of the "unknown" is characterized by comparing the dosages necessary to produce the same effect as a reference compound in the same bioassay. The results on the compound which was tested are expressed as "units of biological activity" as compared to the activity of the standard. This approach is still used to express the potency of some pharmacologic preparations, e.g. the potency of compounds used for the control of

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[20]Quantitative risk assessments do not employ a reference measure, per se, in the calculation. However, the justification for the quantitative risk assessments, similar to the relative potency approach, is the "standardization" of the information obtained from the toxicologic bioassays, regardless of test species, route of exposure, etc., such that all data are on a common scale. Hypothetically, this scale represents a surrogate measure of human risk and tends to be used to directly compare the "risks" associated with exposure to different agents.

diabetes are expressed in terms of "U. S. P. Insulin Units" (the U. S. P. Insulin Reference Standard is used to calibrate the preparations) (Larner, 1980).

Comparable methods are used in toxicologic research, where the approach has been used to standardize experimental results and thereby compare the relative toxicities of compounds (e.g. relative LD50's; relative neurotoxicity). For example, one application of this approach has been used to demonstrate the variations in the toxicity of a variety of dioxin isomers, relative to the 2,3,7,8-TCDD isomer (Bellin and Barnes, 1985). Another example is the evaluation of the "selective toxicity" of particular pesticides by standardizing the results obtained in insects with the rat oral LD50 for the individual compounds (Murphy, 1986).

2.3.1.2 Examples of the application of the relative potency approach to mutagenicity and carcinogenicity bioassays

The use of relative potency approaches has been demonstrated to be of utility in the evaluation of biological experiments--particularly when the endpoints are empirical, poorly understood phenomena. Different types of relative potency techniques have been suggested by a number of investigators for the analysis of mutagenicity and tumorigenicity data in the assessment of human hazard. As demonstrated above, the use of this type of technique to standardize experimental results allows for a more systematic evaluation and application of the data.

For example, the genetic damages produced by gamma radiation has been fairly well characterized. Since the same types of damage can be observed with exposure to some chemicals, it has been proposed that the genotoxicity produced by these chemicals can be standardized by the dose of gamma radiation necessary to produce the same effect. The results of this ratio would be expressed as rad- equivalents.

As with other estimates of toxicity generated using bioassays, the comparison of rad-equivalences is limited to specific genetic effects and experimental conditions (Laterjet et al., 1982; Golkar, 1983). Further, the application of this type of method for the standardization of chemically induced genetic damage is severely constrained by the very different dynamics modulating the effects of radiation and chemical agents.

Although both are complicated, the endpoint of the carcinogen bioassay is more formidable to evaluate and summarize than the mutagenicity bioassay. Reasons for these difficulties have been discussed. In brief, given the limitations and idiosyncratic responses of the carcinogen bioassay, most protocols are simply designed to test for the capacity of a compound to act as a carcinogen. Since the protocols for these bioassays are limited to a few very large doses (generally the MTD and one-half the MTD) there is a limited capacity to directly compare the carcinogenic potencies of different compounds.

To compensate, numerous methods have been established to "standardize" the results. For example, to contrast the potency of a variety of petroleum based tars and oils, Twort and Twort (1930) developed a comparison between the average number of tumors developed in a specific period of time with a hypothetical reference compound. Similarly, Shimkin et al. (1966) used an analysis which employed the ratio of an arbitrarily selected number over the total dose necessary to produce a specific response (i.e., 10,000 / total dose for a positive response). If used properly, these approaches are useful within their experimental context. However, they are not flexible or generalizable methods, i.e., they cannot be used for the analysis and comparison of data generated in other settings or experiments.

A simpler and less restrictive method was developed by Iball (1939). This approach was based on the ratio of the percent of all treated animals with tumors standardized (i.e.,

divided) by the average latency for the appearance of the tumors (percent of treated animals with tumors times 100/average latency period in days). Although the method is limited by its simplicity (all animals must be exposed to equimolar doses of the compounds being compared), it has been used to compare the carcinogenic relative potencies of certain polycyclic hydrocarbons and the effect of different protocols on the mutagenicity of the same compounds (expressed as revertants/nmol) (Tomatis et al., 1982).

Using a Weibull model to estimate the time to median response, Holland and Frome (1984) have also incorporated the element of latency. Data from carcinogenesis bioassays on a variety of petroleum products were standardized relative to a set of data obtained for benzo(a)pyrene. This unique approach to calculating relative potencies also allowed the investigators to evaluate the relationships between latency, the exposure rate, and the probability of tumor occurrence. Since all compounds were tested using the same bioassay conditions, the relative potencies were expressed as a percent of the activity of B(a)P. However, as noted by the authors, this is a very cumbersome technique to use for the calculation of large and diverse sets of data.

Albert et al. (1983) have used a relative potency approach to evaluate the toxicity of diesel particulate emissions in a variety of bioassays (*in vivo* and *in vitro*). Results were analyzed using a "linearized nonthreshold model," similar to the model used by the EPA-CAG for "quantitative risk assessment." As with the calculation of "unit risk", the slopes of the resulting regressions were assumed to represent a quantitative index of potency. These investigators calculated the relative potencies by taking the ratio of the slopes of the dose response curves generated using test and reference compounds in the same bioassays. The results were interpreted to indicate that this application of a relative potency approach was a useful means of evaluating the human hazard associated with exposure to certain types of complex mixtures. However, the application of a linearized model to mutagenicity and carcinogenicity data infers an understanding of the nature of the response that does not appear to be warranted.

Jones et al. (1985, 1987) have developed a relative potency approach which allows for the utilization of a broad spectrum of data. The approach (called RApid Screening of Hazard or RASH) is unusual in that it makes no assumptions about the quality of the data used for the analysis and is not dependent on the use of statistical models. This method is also distinguished by the emphasis on the integration of as much toxicologic information as is readily available.[21]

These investigators have developed extensive rules for the RASH analysis, generally using data obtained from the toxicologic data base RTECS (Registry of Toxic Effects of Chemical Substances). Relative potency values are computed by taking the ratio of doses between "reference" and "test" compounds needed to produce the same effects, in the same test systems ("exact matches"). Data from tumorigenicity experiments were given special attention because the protocols and test data are so complicated. For example, to incorporate

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[21]The obstacles to selecting the "most relevant" data for the determination of potential carcinogenicity and/or mutagenicity were reviewed in section 2.1 of this chapter. In brief, lacking understanding of the factors which can influence the outcome of the bioassays (i.e., the context-dependence of the results) it is generally difficult to defend the use of certain data sets, or statistical models, while rejecting others. These difficulties may be overcome with the development of methods which do not rely on untestable models. In turn, this would allow for more defensible criteria for the selection and analysis of the toxicologic data.

a surrogate of the effect of time in these experiments, it is recommended that RTECS entries should only be compared when treatment times are comparatively close.[22]

When data are too sparse to allow for the "exact matching" between the "test" compound and the "reference" compound of choice ("primary reference"), Jones et al. (1985; 1987) have proposed alternative methods of comparison. The supplementary approaches include the use of "secondary" standards and less stringent criteria ("near" and "reasonable" matches) for the selection of information used to calculate the relative potencies.

2.4 CRITICAL SYNTHESIS OF PREVIOUS LITERATURE

As should now be evident, the actual process of attempting to perform health hazard analysis is fraught with complications, often in spite of a wealth of data in several toxicologic test systems. These dilemmas are imposed by the number of pivotal issues that remain unresolved. Hence, in the absence of more scientifically credible models, the application of toxicologic data for decision making will continue to require a recognized uncertainty.

Part of the reason for the complications is that the different modes of study (epidemiology, tumorigenicity, and mutagenicity bioassays) are based on very different types of assumptions. These differences can lead to the erroneous conclusion that one method of study is more applicable than another. For example, the tighter specifications of the laboratory studies and absence of the innumerable confounding social and environmental issues can cause one to place value on the toxicologic test systems as being more informative. Alternatively, the relevance of species and of the exposure can make the epidemiology appear to be more informative. Nonetheless, it must be emphasized that in most situations these different approaches are most effective when used in a complementary fashion.

Unfortunately, no simplistic "rule-of-thumb" or one-dimensional analysis is possible for such a complex, and socially significant body of knowledge (NRC, 1983). No method taken alone should be recognized as having the capacity to address all of the complex issues that arise in the evaluation of human risk. Alternatively, comprehensive reviews of the quality and quantity of the data can be employed as means of distinguishing comparative or relative estimates of risk (Walsh et al., 1982; Dudney et al., 1983; Interdisciplinary Panel on Carcinogenicity, 1984).

In this way priorities can be established, yet remain flexible and responsive to the respective state of knowledge. At the same time, scientists must accept an obligation to society, and not permit false expectations regarding the quality or accuracy of the information used to develop the risk assessments. It is imperative that approaches employed to evaluate

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[22]It is well recognized that several aspects of time must be considered in the analysis of tumorigenic data; e.g., time from first exposure, time since exposure ended, the "time-to-tumor", and the age of the animal during the exposure period (SGOMSEC, 1985). The RTECS data base does not include information on these variables. Jones et al. (1987) attempt to compensate by using the duration of exposure to the compound. To do this, they have constructed and recommend the use of an algorithm to assist in the "matching" of carcinogenicity experiments. In brief, they suggest that experiments not be matched if: $0.8 < T_1 / T_2 < 1.2$, where T_1 = duration of exposure to the "reference compound" and T_2 = duration of exposure to the "test compound".

the hazard represented by exposure to a compound reflect the actual state of knowledge. Neglecting to explicitly state the inherent limitations of the data can result in gross over or under estimates of hazard and risk, or greatly inflate the concept of accuracy and precision, thereby distorting the purpose of these investigations.

A relative potency framework may help to explicitly state, and perhaps overcome, some of these shortcomings in that the approach is very adaptable. Methods have been developed which are not model dependent, can utilize diverse data, and do not mask deficiencies in the existing data (Jones et al., 1985; 1987). Further, since the approach reduces data to a common scale, it can provide a scientifically justifiable framework for the necessary integration of data and subsequent comparison of the endpoints observed in the toxicologic bioassays (Walsh et al., 1982; Dudney et al., 1983).

3.0 METHODS AND MATERIALS

Chemical carcinogenicity and mutagenicity data were analyzed in an attempt to determine if short-term test systems (bioassays) were capable of predicting the hazard of a compound as estimated using data from chronic test systems (bioassays).[23] The theoretical basis of the analysis, a relative-potency approach, has been reviewed. In brief, the notion of relative potency is based on the standardization of data to a common scale.

3.1 SOURCES OF DATA

Chemicals were selected primarily from the list compiled by the International Agency for Research on Cancer (IARC) (IARC, 1982). This list represents the decisions of an "International Working Group" whose conclusions are widely recognized. A small number of chemcials generally regarded as suspect were also included. Chemicals from the IARC list are organized into categories based upon the "degree of available evidence." The categories developed by IARC include: (1) chemicals that are recognized as human carcinogens (Group I), (2) chemicals that are probably carcinogenic to humans (Groups IIa and IIb), and (3) chemicals that cannot be classified as to their carcinogenicity to humans (Group III).

Fifty-three individual agents were selected, mostly from the categories of "recognized as human carcinogens" (Group I) and "probably carcinogenic to humans" (Groups IIa and IIb). Chemicals were selected to represent a wide variety of chemical classes (e.g., nitrosamines, halogenated hydrocarbons) and theorized mechanisms of action (e.g., direct acting, indirect acting, inorganic metals, and compounds whose carcinogenic mechanisms have not been identified).

It is likely that information on chemicals selected from the IARC's categories is potentially biased---i.e., the chemicals in these categories probably have more data available regarding their toxicity than a chemical not classified by IARC. In turn, an implication of this selection process is that the sample of chemicals used cannot be thought to be a representative sample of the universe of all chemicals (Saracci, 1981). However, a less biased approach to the selection of chemicals for this analysis was beyond the scope of the investigation. A list of the chemicals selected for the study and other pertinent information related to these agents is presented in Appendix A.

A limitation of the IARC compendium of chemical carcinogens is that it does not include a quantitative estimate of potency nor information on the potential mechanism of action. Quantitative information on the chemicals selected for this study was obtained from the Registry of Toxic Effects of Chemical Substances (RTECS). In the public domain, the RTECS data base represents the most extensive, single-source document available for data on chemical mutagenicity and carcinogenicity. The RTECS data base publishes data representing the lowest reported doses that produce toxic effects via a variety of exposure routes and in different species. Obviously, there are numerous limitations associated with the use of this type of data. These include: (1) the lack of dose-response information, (2) that, by law, the Registry only publishes positive information (i.e., a toxic effect had to be produced

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[23]The context dependence of these bioassays has been reviewed. Given the idiosyncratic nature of the experimental results, the term "test system" will be employed to refer to the combination of test species and experimental protocol.

for a citation to appear in the Registry), and (3) that the Registry publishes data that have not been "selected" by an expert committee; i.e., that does not conform with certain pre-selected criteria[24] (Lewis and Sweet, 1985). The lack of "rigor" in the selection of the data published in the Registry has made the use this data base for scientific pursuit controversial.[25]

Nonetheless, from a pragmatic perspective, it was judged that these conditions were acceptable, given the magnitude of the data requirements of the project. Also, it must be understood that the toxicologic bioassays used for the study of the potential carcinogenicity and mutagenicity of a chemical have incorporated a number of biases intended to make the assays as sensitive as possible. Theoretically, for hazard assessment, these features tend to emphasize the significance of a positive effect per se and concomitantly deemphasize the biological significance of other features of the experiment. It is presumed that this bias would generally cause an overestimate of a human health hazard. Further justification for using this non-peer-reviewed data set is the incomplete information regarding objective rules which would allow for the rational acceptance or dismissal of data obtained from diverse test systems. Rules for selection would have to incorporate (1) an understanding of the cell biology, biochemistry, or etiology of the diseases called cancer, and (2) an understanding of the collage of factors which can influence the outcome of a bioassay. Thus, it is difficult to rationalize not incorporating data simply because the investigator did not meet the criteria dictated by an "expert committee" (criteria which obviously can and have been changed) (Task Force of Past Presidents of the Society of Toxicology, 1982; Interdisciplinary Panel on Cancer, 1984). Additionally, given the limited amount of data available for any specific compound and the fact that "expert committees" have not produced methods which allow for a quantitative comparison of mutagenicity and tumorigenicity data, the use of a source of information not widely recognized for its scientific merit may be justifiable.

It is difficult to evaluate the significance of the information not contained in the RTECS data base. The reasons for this include, for example: (1) the organization does not publish negative studies, (2) the information has not been published in the "open literature," or (3) the respective literature search has not yet uncovered the information. Also, in a limited number of cases, test results may not be included because the protocol of an experiment did not meet the inclusion criteria of the organization. Generally, the latter is limited to reports in which the results cannot be interpreted (Lewis and Sweet, 1985).

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[24]Data in the RTECS data base is abstracted from the literature by a company that is subcontracted by the National Institute for Occupational Safety and Health (NIOSH) specifically for this task.

[25]This is a sensitive issue. Some claim that the data in the Registry is not worthwhile because it is not "peer-reviewed" (this term has come to be synonymous with data selection by an "expert committee"). In contrast, one could also argue that since the information has been published in scientific journals it has been "edited," and thereby validated, by the scientific community (Lewis and Sweet, 1985). Hence, a potentially significant feature of this source of data is that it reflects a larger sample of the information in the general scientific literature.

3.2 DESCRIPTION OF DATA BASES CONSTRUCTED FOR THE ANALYSIS

Two data bases were constructed to contain the tumorigenicity and mutagenicity data obtained from RTECS. These represented the "master files" for the analysis.[26]

3.2.1 Structure of the "Tumorigenicity Master File"

The file contained the following information for each experiment in this data base:[27]

1. The RTECS access number for the chemical
2. The Chemical Abstracts Service Registry Number (CAS number) for the chemical
3. The molecular weight of the chemical
4. The type of dose (TDLo, TD)[28]
5. The species used
6. The route of exposure
7. The cumulative dose
8. The units of the dose

Results from experiments on rodents were reported most frequently. Any references to human data (e.g., case studies citing an exposure believed to have produced toxicity in a man, woman, child, or infant) or to unidentified species (e.g., mammal) were deleted. All other data were used without critical review. It should be noted that the RTECS compilation does not incorporate any information on the strain or sex of the test organisms used in the respective experiments.

As noted, the file contained the results of tumorigenicity experiments (records) for 53 carcinogens. There were 658 records, with an average of 13 records per chemical. Ninety percent of the data were from rodent bioassay (45% on rats; 35% on mice; 10% on hamsters). A sample from this file is seen in Appendix C.

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[26]The original citations for the data used in this analysis can be found in the RTECS data base (Lewis and Sweet, 1987).

[27]See Appendix B for details.

[28]These are dose designations employed by RTECS specifically for tumorigenesis data. Normally RTECS only includes the lowest dose necessary to produce an effect in a given test system. However, given the concern for the potential of a compound to act as a human carcinogen, the registry publishes multiple studies in which a tumorigenic response has been reported. The TDLo is used to refer to the lowest dose found to produce an effect; the TD is used for other entries which used the same species-route combination.

3.2.2 Structure of the "Mutagenicity Master File"

The file contained the following information for each experiment reported in this data base:[29]

1. The RTECS access number for the chemical
2. The CAS access number for the chemical
3. The molecular weight of the chemical
4 The species used in the experiment
5. The biological endpoint evaluated
6. The cell type (where appropriate)
7. The route of exposure (where appropriate)
8. The cumulative lowest dose to produce the effect
9. The units of the dose

This file contains information generated on a wide variety of short-term test systems. These represent mutation data for both *in-vitro* and "limited" whole-animal investigations. For example, these include bacteria, molds, yeasts, insects, *in-vitro* mammalian cell cultures, and the results of intact animal studies.

As with the *in-vivo* data, all nonspecific references (e.g., unidentified microorganisms, unidentified cell types) were deleted. Also, experiments which had the units of dose reported incompletely (e.g., only the amount of the compound administered was given, not information on the volume to which it was applied) were deleted. All other data were used without critical review.

It should be noted that the RTECS citations on the mutation test systems tend to be generic. For example, data from experiments using the *Salmonella typhimurium* reverse mutation assay ("Ames Assay") are limited to acknowledgment of the species---no mention is made of the strain. Also, *in-vitro* bioassays frequently use a "biochemical (or microsomal) activation system" prepared from liver homogenate for enzymatic activation of promutagens. The information in the RTECS data base is limited to the presence or absence of an activation system. No details are given on the experimental protocol for the preparation of the homogenates; neither the species or sex of the organism from which the homogenates were prepared is listed, nor is the inducing agent identified.

This file contained the results of mutagenicity experiments for the same 53 carcinogens. The file had 2140 records, with an average of 41 per chemical. A sample of this file is seen in Appendix D.

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[29]See Appendix B for details.

3.3 ANALYSIS

To address the research question, the study was divided into three distinct phases. Phase one concentrated on the calculation of the potencies of the test chemicals relative to the reference chemicals. The results of these calculations were summarized in phase two, and the correlation between the results obtained from short-term and chronic bioassays was tested in phase three.

3.3.1 Phase One of the Analysis

This portion of the analysis was dedicated to the transformation of the data in the "master files" to reference-chemical-specific values, using a modification of the relative-potency framework developed by Jones et al. (1985; 1987). As noted in Sect. 2.3, this type of calculation theoretically allows for a standardization of a variety of measures. Given the nature of this analysis (standardizing the measures of biologic activity using the activity of reference compounds in the same bioassays), it was foreseeable that a random or systematic error could influence the results. To reduce the possibility for error that could be attributed to the use of a single or limited number of reference compounds, this series of calculations was replicated with 14 reference compounds. Chemicals selected as references were purposefully chosen to represent a wide variety of chemical classes and hypothesized mechanisms of action (MOA). The compounds used as references are shown in Table 3.

The details of the relative-potency approach have been discussed. The approach used in this analysis entailed a modification of the technique described by Jones et al. (1985, 1987). The general equation for the calculation of relative potency (RP) is:

$$RP = \frac{\text{dose of the reference chemical in assay A}}{\text{dose of the test chemical which produces the same type and level of biological effect in assay A}}$$

The procedures used to apply this approach to the data obtained from the RTECS data base are described in the following sections.

3.3.1.1 Calculation of tumorigenic relative potencies

The method used to calculate tumorigenic relative potencies is outlined below.

1. All doses were converted to millimolar units.

2. The experimental parameters used to "match" the entries were a combination of the type of dose (TDLo, TD), test species, and the route of exposure.[30] Examples include:

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[30]Duration of the experiment, as a surrogate of the time-to-tumor, was not incorporated into this calculation. The significance of this is reviewed in the Discussion (Section 5).

Table 3. Reference compounds used in analysis

Chemical name	MOA[a]	MUTREC[b]	TUMREC[c]
Methylmethane sulfonate	Direct	226	10
N-methyl-n-nitrosourea	Direct	149	51
Propiolactone	Direct	70	18
Benzene	Epigenetic	55	17
Epichlorohydrin	Epigenetic	39	13
DES	Hormone	47	33
2-Napthylamine	Indirect	45	24
3-MC	Indirect	80	53
B(a)P	Indirect	207	44
Cyclophosphamide	Direct	139	18
DMNA	Indirect	138	33
Cadmium chloride	Metal	47	12
DDT	Promoter	26	15
TCDD	Promoter	19	11

[a] Theorized biochemical mechanism of action. Information on the theorized mechanism of action of the individual agents was obtained from Klaassen et al. (1986). These classifications are not meant to be definitive. For example, some investigators classify cyclophosphamide as an indirect acting agent (Connor, 1987).

[b] Number of mutagenicity experiments (records) listed in the RTECS data base.

[c] Number of tumorigenicity experiments (records) listed in the RTECS data base.

a. The test and reference compounds were orally administered to the rat, and the effect, for each chemical, was categorized as a TD.

b. The test and reference compounds were intraperitoneally (ipr) administered to the guinea pig, and the effect, for ea... chemical, was categorized as a TDLo.

c. The test and reference compounds were intratrachially (itr) administered to the dog, and the effect, for each chemical, was categorized as a TD.

3. After tumorigenicity assays for reference and test chemicals were "matched," the ratio of the reference and test doses was calculated (reference dose/test dose).[31] The result of this calculation is the tumorigenic relative-potency value for the test compound relative to a reference compound in the particular bioassay. Examples of this type of calculation are seen in Table 4.

3.3.1.2 Calculation of mutagenic relative potencies

The method used to calculate mutagenic relative potencies is outlined below.

1. All doses were converted to millimolar units.

2. The experimental parameters used to "match" the data included a combination of the biological effect and the test-system in which it was observed. The presence or absence of an enzymatic activation system, cell type, or route of exposure was also included where appropriate. For example:

   a. The test and reference compounds produced mutation in *salmonella* (sat) in the presence of an activation system (mma).

   b. The test and reference compounds produced mutation in *saccharomyces cerevisiae* (smc) (yeast) without an activation system (mmo).

   c. The test and reference compounds produced an oncogenic transformation (otr) in hamster (ham) embryo cells (emb).

   d. The test and reference compounds produced DNA damage (dnd) observed in cultured rat (rat) liver cells (liv).

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[31]The relative-potency values were calculated using the software package dBase III Plus (Ashton-Tate, copyright 1985) and an IBM Personal Computer (IBM PC- XT). Programs were written in the dBase III programming language and were designed to perform the relative-potency calculations using a specified reference compound with either the entire tumorigenicity or mutagenicity master file.

Table 4. Example of tumorigenicity relative-potency calculations[a]

Compound	Route	Endpoint	Species	Dose (mmol/kg)	Relative potency
Reference	Oral	TD	Rat	5.00	
Test	Oral	TD	Rat	10.00	0.50
Reference	Intra-peritoneal	TDLo	Guinea Pig	0.23	
Test	Intra-peritoneal	TDLo	Guinea Pig	57.00	0.004
Reference	Intra-tracheal	TD	Dog	0.187	
Test	Itntra-tracheal	TD	Dog	54.00	0.003

[a] This table is used simply for heuristic purposes. The data for both the reference and the test compounds are hypothetical. For examples of the actual results see Appendix E.

e. The test and reference compounds were intraperitoneally administered (ipr) to mice (mus) and produced sister chromatid exchange (sce).

3. Comparable mutagenicity assays for reference and test chemicals were "matched," and the ratio of the reference and test doses was calculated (reference dose/test dose).[32] The result of this calculation is the mutagenic relative-potency value for the test compound relative to a reference compound using a particular bioassay. An example of these calculations is seen in Table 5.

As demonstrated in Tables 4 and 5, when a test compound is "standardized" to a reference compound, an array of relative-potency values are generated. However, there is no systematic, empirical confirmation that the evidence obtained from any one test system[33] is more or less relevant than any other. Hence, it was assumed that all experimental interactions of test organisms and a chemical provide equally useful information about the potential for human toxicity (Freireich et al., 1966; Purchase, 1980a; DuMouchel and Harris, 1983).

In this context, the distributions of values are taken to portray "biologic activity profiles" or "arrays" of values representing the variation in biological activities of "test" compounds, standardized to (or relative to) "reference" compounds. In other words, data from the short-term tests (which are theoretically modeling mutagenic potency in human somatic cells) were pooled to develop an aggregate measure of mutagenicity. Similarly, data from the chronic bioassays (theoretically modeling the capacity of a compound to act as a human carcinogen) were pooled to develop an aggregate measure of tumorigenicity. Thus, the ranges of values observed using this type of an approach are viewed as being very informative (Jones et al., 1985,1987).

3.3.2 Phase Two of the Analysis

This phase of the analysis summarized the results generated in phase one. The median value of an "array" of values, illustrating the potency of a test chemical relative to a reference chemical, was taken to be the most appropriate summary statistic of the central biologic tendency. This statistic was utilized because the parent or underlying distribution of the "array" is unknown. The median value is also useful in that it is insensitive to outliers in the distributions. This fact alleviates some of the concerns about errors in the RTECS database. The calculation was restricted to those "arrays" which had three or more values (it was assumed that less than three values would probably not provide a useful estimate).

The interquartile range was used as the most practical estimate of the uncertainty and was summarized in a single value as the ratio of the highest and lowest values of the range (e.g., highest value in interquartile range/lowest value in the interquartile range). The median relative-potency values and ranges for each of the test chemicals were entered into reference chemical-specific files.

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[32]See footnote 30 for details.

[33]The term "test system" is used to allude to the "context dependence" of a given experimental outcome and thereby serves to incorporate the idiosyncracies of the interaction between the test organism and the exposure conditions.

Table 5. Example of mutagenicity relative-potency calculations[a]

Compound	Cell Type/ Route	Endpoint	Species	Dose	Relative potency
Reference	mma	sal		0.12	
Test	mma	sal		0.38	0.32
Reference	mmo	smc		0.081	
Test	mmo	smc		0.15	0.54
Reference	emb	otr	ham	0.0011	
Test	emb	otr	ham	0.050	0.02
Reference	liv	dnd	rat	0.15	
Test	liv	dnd	rat	0.60	0.25
Reference	ipr	sce	mus	0.0039	
Test	ipr	sce	mus	0.00010	39.00

[a] As with Table 4, this table is used simply for heuristic purposes. The data for both the Reference and the Test compounds are hypothetical. For examples of the actual results see Appendix F.

3.3.3 Phase Three of the Analysis

This phase of activity was dedicated to answering the research question: Are the results obtained from test systems measuring mutagenicity predictive of the results obtained from test systems measuring tumorigenicity? To address this question, the correlation between the median tumorigenic relative potencies and median mutagenic relative potencies of the test chemicals was evaluated. Since assumptions regarding the underlying distributions were not warranted, a nonparametric Spearman Rank Correlation Coefficient [R_s] was calculated (Snedecor and Cochran, 1982; Steel and Torrie, 1980).

Spearman's coefficient of rank correlation (Spearman's Rho or [R_s]) is used to evaluate the concordance in the ranking of the values of two variables, X and Y, and cannot be interpreted as a measure of linear correlation. The procedure for the calculation is as follows (Steel and Torrie, 1980):

1. The data are ranked for each variable.

2. Differences in rank are calculated for the paired observations (D).

3. Estimates of the Spearman coefficient of rank correlation [R_s] between variables are calculated using the equation

$$[R_s] = 1 - 6\sum D^2/[n(n^2-1)],$$

where n is the number of pairs.

Hence, the goal of this analysis was to evaluate the consistency of the rank ordering of the median tumorigenic relative potencies and median mutagenic relative potencies of the test compounds in the 14 reference-compound-specific files. The null and alternative hypotheses were

Ho: X and Y are mutually independent
Ha: X and Y are not mutually independent,

where X = the median tumorigenic relative potencies and Y = the median mutagenic relative potencies (where both are standardized to the same reference compound). After ranking X, given the hypothesis of no correlation, it is assumed that Y is drawn at random from "n" factorial permutations of the possible ranks. The rank correlation can range between +1.0 (complete concordance) to -1.0 (complete discordance) (Snedecor and Cochran, 1982). The estimates of the Spearman Correlation Coefficient were calculated using the statistical software package P.C.- S.A.S. (S.A.S. Institute, Inc., copyright 1985).

To test the null hypothesis of no correlation, the estimate of Spearman's Rho [R_s] was converted to a t statistic. According to Steel and Torrie (1980), the criterion

$$t = [R_s] \left(\frac{n-2}{1-[R_s]^2}\right)^{1/2}$$

is approximately distributed as Student's t with n-2 degrees of freedom (where n is the sample size---i.e., the number of pairs). The t statistic was tested against a two-tailed t distribution to detect any departure from independence (Daniel, 1978; Snedecor and Cochran, 1982).

4.0 RESULTS

As the research was composed of three distinct phases, results from each of these phases will be discussed in turn.

4.1 PHASE ONE OF ANALYSIS

This phase of the research focused on the calculation of the relative potencies of the test chemicals relative to each of the reference chemicals. This was accomplished by "matching" the bioassays which were used to experimentally describe the activity of "test" compounds and "reference" compounds. The ratio of the dose of the reference agent divided by the dose of the test agent found to produce the same effect, in the same test system, is defined as the relative potency for that test compound. As with the radiobiological and pharmacological applications of this type of approach, this procedure is thought to standardize the data relative to the reference. The results of this effort were "arrays" or "biological activity profiles" for each of the test compounds relative to each of the individual reference compounds. It should be noted that each of the "biological activity profiles" for the pairings between the reference chemicals and test chemicals is unique. Each is distinguished in size (e.g., some "arrays" had in excess of 100 entries, others were limited to very few) and nature of the constituents (e.g., as might be expected, different bioassays are seen in different arrays). These differences are dictated by the type and number of bioassays which "matched" for the specific reference and test compound combinations.

The various combinations of the 14 "reference" compounds and 52 test compounds produced approximately 700 individual "arrays" for both the tumorigenicity and mutagenicity data (1400 total). Examples of this work are seen in Appendix E for the tumorigenicity data and Appendix F for the mutagenicity data.

4.2 PHASE TWO OF THE ANALYSIS

Lacking information to indicate the superiority or greater relevance of one experimental setting (i.e., test species, protocol) over another, it was assumed that all information was of equal value. Hence, the series of relative-potency values in the individual arrays were taken to represent distributions reflecting the biological activity of a compound as measured in a variety of different, but conceptually equal, for hazard ranking, test systems. Since the underlying statistical distributions of relative-potency values are not known nor are readily identifiable, results were summarized by calculating the median value and interquartile range for each array which had three or more relative-potency values.

The product of this phase was 28 reference chemical-specific tables (14 for the mutagenicity data and 14 for the tumorigenicity data). These tables were used to organize the median relative potencies, and their respective interquartile ranges, of the 52 test chemicals relative to each of the reference chemicals. Zero was entered for all arrays which had less than three values. An example of this work is seen in Appendix G.

4.3 PHASE THREE OF THE ANALYSIS

To determine how well the measures of mutagenicity predicted the measures of tumorigenicity, the median mutagenic and median tumorigenic relative potencies were tested for correlation. This analysis was limited to mutagenicity and tumorigenicity data standardized to the same reference compound. As noted, to minimize the effect of random or systematic errors that could be attributable to the choice of a limited number of reference compounds, the relative-potency calculations were performed using 14 reference agents. Thus, the tests for correlation were calculated on the results obtained by standardizing the tumorigenicity and mutagenicity data to the data associated with each of the 14 reference agents. Again, this was done to minimize the potential for spurious results that could be attributed to the chemical class or mechanism of action of the reference compounds.

It was not possible to calculate a median relative potency for all 52 of the test compounds relative to all of the reference compounds because of incomplete overlap of the test systems cited in the Registry of Toxic Effects of Chemical Substances (RTECS). This resulted in slight variations in the nature of the test compounds (number and identity) which were evaluated per reference compound. The lists of compounds used in each analysis, their median tumorigenic and mutagenic relative potencies, and their interquartile ranges are seen in Appendix H.

The underlying distributions of the data have not yet been investigated, nor are theoretical arguments available to justify convenient assumptions regarding the shapes of these distributions. This constrained the statistical analysis to be nonparametric: a Spearman Rank Test was selected to evaluate the relationship. This type of an approach is designed to evaluate the consistency of the rank order of the measures being compared (i.e., the rank order of the median mutagenic relative potencies of a series of test compounds relative to a reference vs. the rank order of the median tumorigenic relative potencies of the same compounds relative to the same reference). The results are presented in Table 6.

It can be seen in Table 6 that, for most of the reference agents, the rankings of median mutagenic potencies is highly correlated with the ranking of median tumorigenic relative potencies. Only the data standardized to cadmium ($p = .06$), 2-napthylamine ($p = .08$), DDT ($p = .16$), and TCDD ($p = .61$) were not statistically significant at the level generally accepted for significance ($p < .05$). Nonetheless, it is apparent that all correlations were positive, and thereby in the same direction.

Given the conservative summary statistic (tested against a two-tailed distribution), the consistency of the results, and the strength of correlation observed for most of the reference compounds, the result observed for the data standardized to TCDD is clearly anomalous. Indeed, in contrast to the rest of the data, the lack of correlation is quite profound ($p = .61$). It was postulated that this observation may be the result of unique characteristics of this data set which cause it to be substantially different than the data standardized to the other reference compounds.

Table 6. Comparison of ordering of median mutagenic and median tumorigenic relative potencies

MOA[a]	Reference compound	RHO[b]	N[c]	P values[d]
Direct	Methylnitrosourea	0.407	33	p = .02
Direct	Cyclophosphamide	0.493	30	p = .005
Direct	Propiolactone	0.447	33	p = .02
Direct	Methylmethane sulfonate	0.407	27	p = .04
Indirect	Dimethylnitrosamine	0.639	44	p = .0001
Indirect	Benzo(a)pyrene	0.416	36	p = .01
Indirect	3-methylcholanthrene	0.376	43	p = .01
Indirect	2-napthylamine	0.301	34	p = .08
Epigenetic	Epichlorohydrin	0.427	31	p = .02
Epigenetic	Benzene	0.421	31	p = .01
Epigenetic	DES	0.373	29	p = .04
Epigenetic	DDT	0.309	22	p = .16
Epigenetic	TCDD	0.111	22	p = .61
Metal	Cadmium	0.425	20	p = .06

[a]Theoretical mechanism of action of reference compound. See Table 3 for details.

[b]Spearman Rank Correlation Coefficient.

[c]Number of compounds which had sufficient information for analysis.

[d]The correlation coefficient was transformed to a t statistic and tested against a two-tailed distribution. See Sect. 3 for details.

In order to characterize the properties of the data standardized to TCDD, a subsample of the data was examined in greater detail. In contrast to the preceding analysis (which compared the ranking of the potency of compounds as measured by different endpoints but using the same reference compound), this analysis was performed by correlating data reflecting the same endpoints but was standardized by different reference agents (e.g., ranking of median relative potencies for tumorigenicity data using one reference vs. the ranking of the median relative potencies for tumorigenicity data of the same compounds, with a different reference agent). To maximize the possibility of observing any trends, all combinations of the data standardized to six reference compounds were tested for correlation.

The reference chemicals were purposefully selected to be a representative subsample of the agents used in the original analysis. As with the original tests for correlation, the approach employed a series of Spearman Rank Tests. The conceptual basis of this procedure was that, in theory, if the ranking of the test compounds standardized to TCDD was genuinely distinct in contrast to the ranking of the test compounds relative to the other reference agents, the reason for the anomaly might become evident under closer scrutiny. The reference compounds, their respective mechanisms of action, and pertinent statistical information are seen in Table 7 for the tumorigenicity data and Table 8 for the mutagenicity data.

It was hoped that this stratagem would help to isolate novel characteristics of the data standardized to TCDD, which distinguished it from the data standardized to the other reference compounds. The results of the exercise were very revealing. As seen in Tables 7 and 8, the rank ordering of the test compounds standardized to each reference agent was correlated with the rank ordering of the test compounds standardized to five other reference agents. Despite the dissimilarity of the reference agents, the 15 sets of correlations on the tumorigenicity data were highly significant ($p = .0001$ in all cases). Similarly, while the results on the mutagenicity data did not demonstrate the same degree of consistency in the level of statistical significance, most of these correlations were also significant.

The results of the analysis with the mutagenicity data standardized to TCDD are distinguished from the rest of the data. Although some of the correlations with TCDD were statistically significant (i.e., TCDD vs. DMNA, TCDD vs. benzene, and TCDD vs. epichlorohydrin), others were extremely pronounced in their lack of correlation (i.e., TCDD vs. propiolactone, $p = .44$, and TCDD vs. cadmium, $p = .41$). Since virtually all of the other correlations were statistically significant, the anomaly observed in the original analysis (i.e., the lack of correlation between tumorigenicity and mutagenicity using data standardized to TCDD) appears to be explained by attributes of some of the mutagenicity data standardized to TCDD.

Table 7. Correlation of tumorigenicity data using different references[a]

	Propiolactone (Direct)	DMNA (Indirect)	Benzene (Epigenetic)	TCDD (Epigenetic)	Epichlorohydrin (Epigenetic)	Cadmium (Metal)
Propiolactone (Direct)	---	---	---	---	---	---
	---	---	---	---	---	---
DMNA (Indirect)	N[b] = 36	---	---	---	---	---
	p < .001[c]	---	---	---	---	---
Benzene (Epigenetic)	N = 34	N = 36	---	---	---	---
	p < .001	p < .001	---	---	---	---
TCDD (Epigenetic)	N = 31	N = 35	N = 32	---	---	---
	p < .001	p < .001	p < .001	---	---	---
Epichlorohydrin (Epigenetic)	N = 32	N = 34	N = 34	N = 32	---	---
	p <.001	p < .001	p < .001	p < .001	---	---
Cadmium (Metal)	N = 20	N = 22	N = 22	N = 17	N = 19	---
	p < .001	p < .001	p < .001	p < .05	p < .001	---

[a]References have been selected to represent members of the major classes of theorized mechanisms of action.

[b]Number of compounds which had sufficient information for analysis.

[c]As with the other tests of correlation, the Spearman Rank Correlation Coefficient was transformed to a t statistic and tested against a two tailed distribution.

Table 8. Correlation of mutagenicity data using different reference[a]

	Propiolactone (Direct)	DMNA (Indirect)	Benzene (Epigenetic)	TCDD (Epigenetic)	Epichlorohydrin (Epigenetic)	Cadmium (Metal)
Propiolactone (Direct)	---	---	---	---	---	---
	---	---	---	---	---	---
DMNA (Indirect)	N[b] = 41	---	---	---	---	---
	p < .001[c]	---	---	---	---	---
Benzene (Epigenetic)	N = 37	N = 41	---	---	---	---
	p < .001	p < .001	---	---	---	---
TCDD (Epigenetic)	N = 30	N = 32	N = 32	---	---	---
	p < .5	p < .05	p < .005	---	---	---
Epichlorohydrin (Epigenetic)	N = 37	N = 39	N = 35	N = 31	---	---
	p < .001	p < .001	p < .001	p < .001	---	---
Cadmium (Metal)	N = 36	N = 39	N = 36	N = 30	N = 36	---
	p < .025	p < .01	p < .025	p < .05	p < .05	---

[a]References have been selected to represent members of the major classes of theorized mechanisms of action.

[b]Number of compounds which had sufficeitn information for analyis.

[c]As with the other tests of correlation, the Spearman Rank Correlation Coefficeint was transformed to a t statistic and tested against a two tailed distribution.

5.0 DISCUSSION

A combination of a fear of chemically induced cancer and an enhanced public awareness of the capacity to "screen" for carcinogens has made carcinogenicity and mutagenicity studies a standard activity. However, a general lack of understanding of the limitations and deficiencies of the toxicologic test systems, even among "experts," has also produced a rush to legislate solutions for apparent hazards. As a consequence, in many regulatory settings toxicologic data tends to be utilized by focusing on the results of single experiments.

Part of the impediment in applying toxicologic data to hazard assessment is the difficulty of demonstrating unequivocal proof that the agent is a carcinogen, even with chronic bioassay data. This is due in part to the fact that the mechanisms responsible for the production of the disease remain conjectural. Further, substantial evidence indicates that the outcome of the bioassays is context dependent (Gori, 1980; Cumming, 1985). Since the test systems tend to be biased to produce an effect (which is of utility in studying the processes of disease), it is not implicitly clear how to best use this information for hazard assessment (OSTP, 1985).

The dependence on protocol implies that the more a compound is studied, the more likely it will be found to be a carcinogen or mutagen in some experimental setting. Thus, given the limitations of our understanding of the factors which can influence the outcome of these experiments, the use of individual studies does not appear to be a prudent approach. Nor is there widely accepted theory to delineate how to best apply the test results to the human population. In particular, the current tendency to use data obtained from rodent bioassays done at or near the maximum tolerated dose (MTD) to calculate the risks to humans at low levels of exposure is not scientifically credible (Doll and Peto, 1981; Peto, 1981a; Purchase, 1985b; Ames et al., 1987).

Other complications in the use of these data for hazard assessment are arising in the form of protocols being developed which enhance the sensitivity of the respective test systems. This allows for increasing numbers of chemicals--"synthetic" and "natural"--to be recognized as possessing some capacity to act as a carcinogen and/or as a mutagen. Lacking a consensus on the validity of these data, the scientific community wavers between an uncritical acceptance and a diffuse discontent about how to use the results to develop predictions about human hazard (Tomatis, 1977; Epstein, 1978; Efron, 1984; Clive, 1985; OSTP, 1985). However, while toxicologic data cannot serve as a means for the direct calculation of human risk, neither should it be ignored (Ames et al., 1987; Task Force of Past Presidents of the Society of Toxicology, 1982; Doll and Peto, 1981).

The most appropriate use of these data is accompanied by the recognition that the toxicologic test systems are simply biological models, limited in context, and can frequently be manipulated to produce a desired effect. Further, the experimental results can also be influenced by a wide variety of poorly understood endogenous and exogenous modifying variables. Test systems are so sensitive to these factors that they can be used to increase or decrease the probability of a preselected outcome. For the toxicologic data to be used in a scientifically defensible manner, this information must be explicitly incorporated into the schema of hazard assessments.

Much of the controversy has been related to the implications of the data obtained from "short-term" tests. These tests represent a fairly recent development in the testing of potential mutagens (and by default, potential carcinogens) and represent one of the most

important achievements in modern toxicology. The significance of these tests in helping to elucidate potential mechanisms of genetically induced disease is unequivocal. Nonetheless, this is a vastly different activity from hazard assessment, where the goal is to estimate the likelihood of the compound to produce human disease.

One way this issue could be partially resolved would be to demonstrate a correlation between the results obtained from the chronic and the short-term bioassays. While this does not resolve the critical issue of extrapolation to the human population, it provides supportive evidence of human hazard by demonstrating consistency of response in other species and across levels of biological organization (Purchase, 1980a; Walsh et al., 1982; Dudney et al., 1983; DuMouchel and Harris, 1983). Many investigators have attempted to demonstrate this correlation but, in general, have limited their investigations to the study of individual experiments. For limited classes of chemicals this approach has proved to be a useful tool (Rinkus and Legator, 1979). However, the excessive reliance on single studies may have contributed to the lack of generalizable success of most of these studies.

There is no evidence to indicate that any given test system offers more valid measures than other types of bioassays (Purchase, 1980a; DuMouchel and Harris, 1983). Yet, by default, the aforementioned investigations were generally attempting to demonstrate correlations between estimates of specific types of genetic damage observed in *in-vitro* bioassays (e.g., point mutation in the Ames Assay) and a questionable standard (e.g., results from long-term bioassays). Predictably, this type of approach--which customarily employs qualitative evidence (i.e., positive and negative results)--inevitably produces evidence of "false positives" and "false negatives" (Brusick, 1983). Hence, the procedure does not serve as a useful means of validating the use of the short-term test systems nor can it offer the type of evidence that can be used to set priorities.

5.1 REVIEW OF RESULTS

The basis of this investigation was to employ a relative-potency approach (modified from Jones et al., 1985; 1987) which transformed the results of the bioassays to a quantitatively comparable scale. This simultaneously allowed for the aggregation of data and permitted the calculation of an index of potency which reflected the activity of a chemical in a variety of bioassays. Thus, a number of biologic models--reflecting the capacity of a compound to produce a particular endpoint (i.e., mutagenicity or carcinogenicity)--were used to contribute to a composite perspective of the potency of a compound. The results of the study, which focused on the compounds recognized by IARC (1982) to be carcinogens in humans and/or test animals, are intriguing.

Both test and reference chemicals were purposefully selected to represent diverse chemical classes and vastly different mechanisms of action. Despite the enormous differences in the compounds, statistically significant correlations were generally observed between the rankings of median mutagenic and median tumorigenic relative potencies. This was somewhat astonishing given the rudimentary nature of the data employed in the analysis (e.g., no information on variables known to influence the outcome of these bioassays such as dose rate, time to tumor, and "target dose" for the chronic bioassays; no information on the bioactivation systems used in the *in-vitro* bioassays). This correlation was demonstrated using a wide variety of test compounds (a possible 52) and 14 reference compounds. A profound lack of statistical significance was observed only when the test compounds were standardized to TCDD.

In an attempt to elucidate why the data standardized to TCDD was unique, data standardized to six of the reference compounds (including TCDD) was used as a subsample for more detailed study. The purpose of this evaluation was to determine if there was some distinctive characteristic of the data standardized to TCDD in contrast to data standardized to the other reference compounds. This was evaluated by comparing the intraclass ranking of tumorigenic and mutagenic median relative potencies--i.e., the ranking of mutagenic or tumorigenic relative potencies of the test compounds when the data are standardized by different references. Remarkably, the ranking of the carcinogenic relative potencies of all test compounds was nearly identical regardless of the reference used to standardize the data ($p < .001$ in all trials).

Comparisons of the ranking of the mutagenic relative potencies for the test compounds, while not as dramatic as the results for the tumorigenicity data, were generally statistically significant. Correlations that were not significant were limited to those which included mutagenicity data standardized to TCDD. The consistency of the results obtained with the other reference compounds appears to indicate that a peculiar ranking of the test compounds is obtained when particular short-term test systems are standardized to TCDD. No attempt was made to establish which short-term test systems were contributing to these anomalous results.

The correlations observed between the ranking of the mutagenic and tumorigenic relative potencies contributes supportive evidence to the "somatic mutation theory of cancer"--i.e., that mutagenicity plays a critical role in the production of cancer. More specifically, the results appear to support the notion that DNA is a primary, or at least critical, target in the production of cancer. However, a caveat is in order: it must be recognized that, because of the "ecologic" methods employed in the current investigation, the results do not provide evidence that mutation is causally related to the production of malignant transformation.

Another significant finding of this research is the extreme range of median relative potencies observed for the different test compounds. A minimum of approximately a million-fold difference was seen between the most potent and least potent carcinogens and mutagens. This characteristic was observed for the data standardized to all references. Similar observations of a wide variation in the estimates of tumorigenic and mutagenic potencies of different chemicals have also been made by other investigators (Meselson and Russell, 1977; McCann and Ames, 1977; Parodi et al., 1982; Anderson et al., 1983; Gold et al., 1984).

The relevance of this observation is that, despite the enormous range of median relative potencies, the approach demonstrated the capacity to yield consistency in the ranking of the hazard represented by the test compounds used in this investigation. Significantly, this capacity is independent of the reference compound used to standardize the data and of using results from either short-term or long-term bioassays (other than the mutagenicity data standardized to TCDD). It is also of interest to note that agents theoretically acting by "epigenetic" mechanisms were found to be among the most hazardous substances.

5.2 SUMMARY OF RESULTS

In conclusion, the integral feature of this investigation was the use of a relative-potency approach to reduce very rudimentary data to a common scale. This allowed for the subsequent aggregation of information reflecting similar measures of a compound to produce effects (mutation or tumor) in different test systems. Since the compounds used in this analysis (test and reference) represented a wide variety of chemical classes and mechanisms

of action, consideration of these features and other descriptive variables about the experimental parameters may not be necessary for hazard assessment of carcinogens and/or mutagens.

As with any analysis of biomedical data generated on nonhuman species (using protocols designed to maximize the sensitivity of the bioassay), the lack of information on the validity of the biological models limits the capacity to extrapolate the results. Hence, the estimates of the hazard posed by these agents cannot be thought to be representative of a definitive measure of either human hazard or risk. However, given the consistency of the results between the short- and long-term test systems, it is submitted that this type of an approach offers more reliable information about human hazard than the use of single experiments.

While the results of this investigation must be repeated using other sources of data, they offer preliminary evidence that it may be possible to develop novel applications for the short-term tests. These could include, for example, the construction of a predictive battery consisting of a few selected short-term tests. This type of a battery, when standardized to a reference agent and analyzed properly, would be useful in estimating a "rank" or "comparative estimate" of the hazard represented by an unknown substance (or complex mixture) in the context of measures of other known hazards. In the absence of insight into the biological mechanisms leading to disease, or when limited toxicity information is available, this type of an approach could offer rapid guidance in regulatory activities involving potential exposures to large numbers of compounds and complex mixtures.

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APPENDIX A

MASTER LIST OF COMPOUNDS USED IN THE ANALYSIS

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Chemical Name	CAS[a]	RTECS[b]	Hypothesized Mecahnism of Action	Status	Number of Mutation Records	Number of Tumorigenic Records
2-Napthylamine	91598	QM2100000	Indirect	Reference	45	24
3-Methylcholanthrene (3-MC)	56495	FZ3675000	Indirect	Reference	80	53
4-Aminobiphenyl	92671	DU8925000	Indirect		28	12
Acrylamide	3688537	AS3500000	Indirect		46	11
Acrylonitrile	107131	AT5250000	Indirect		29	5
Aflatoxin	1402682	AW5950000	Indirect			
Aldrin	309002	IO2100000	Indirect		8	4
Aniline	62533	BW6650000	Indirect		10	1
Azothioprine	446866	UO8925000	Indirect		26	14
Bischloromethylether (BCME)	542881	KN1575000	Direct		5	13
Benzene	71432	CY1400000	Epigenetic	Reference	55	17
Benzidine	92875	DC9625000	Indirect		54	10
Butylatedhydroxyanisole (BHA)	25013165	SL1945000	Epigenetic		2	9
Butylatedhydroxytolue (BHT)	128370	GO7875000	Epigenetic		6	5
Benzo(a)pyrene [B(a)P]	50328	DJ3675000	Indirect	Reference	207	44
Cadmium Chloride	10108642	EV0175000	Metal	Reference	47	12
Carbon Tetrachloride	56235	FG4900000	Epigenetic		8	10
Chlorambucil	305033	ES7525000	Epigenetic		8	16
Chlordane	57749	PB9800000	Epigenetic		4	2
Chloroform	67663	FS9100000	Epigenetic		16	8
Cis-platin	15663271	TP2450000	Direct		88	3
Cyclophosphamide	50180	RP1759500	Direct	Reference	139	18
Dibromochloropropane	96128	TX8750000	Indirect		20	14
DDT	50293	KJ3325000	Epigenetic	Reference	26	15
Diethylstilbestrol (DES)	56531	WJ5600000	Epigenetic	Reference	47	33
Dichlorobenzidine	91941	DD0525000	Epigenetic	Reference	6	9
Dimethylcarbamoyl Chloride	79447	FD4200000	Direct		26	8
Dimethylsulfate	77781	WS8225000	Direct		43	3
Dimethylnitrosamine (DMNA)	62759	IQ9525000	Indirect		138	33
Ethylyenedibromide (EDB)	106934	KH9275000	Indirect		38	16
Endrin	72208	IO1575000	Epigenetic		2	0

Master List of Compounds Used in the Analysis (Continued)

Chemical Name	CAS[a]	RTECS[b]	Hypothesized Mecahnism of Action	Status	Number of Mutation Records	Number of Tumorigenic Records
Epichlorohydrin	106898	TX4900000	Epigenetic	Reference	39	13
Estradiol	50282	KG2975000	Indirect		19	17
Ethylene Oxide	75218	KX2450000	Direct		33	10
Ethyleneimine	151564	KX5075000	Direct		41	4
Formaldehyde	20000	LP8925000	Indirect		62	13
Hydrazine	302012	MU7175000	Indirect		23	6
Methyliodide	74884	PA9450000	Direct		10	2
Methylmethane Sulfonate	66273	PB2625000	Direct	Reference	226	10
Nickel Subsulfide	12035722	QR9800000	Metal		7	23
N-Methyl-N-Nitrosourea	684935	YT7875000	Direct	Reference	149	51
N-Nitrosonornicotene	16543558	QS6550000	Indirect		4	16
N-Nitrosopiperidine	100754	TN2100000	Indirect		20	19
Phenacetin	62442	AM4375000	Indirect		15	12
Phenobarbitol	57307	CQ7000000	Epigenetic		3	2
Phorbol Acetate	16561298	QH4377000	Epigenetic		40	15
Propiolactone	57578	RQ7350000	Direct	Reference	70	18
Reserpine	50555	ZG0350000	Epigenetic		7	7
Saccharine	81072	DE4200000	Epigenetic		14	3
TCDD	1746016	HP3500000	Epigenetic	Reference	19	11
Thioacetamide	62555	AC8925000	Epigenetic		18	12
Thiourea	62566	YU2800000	Epigenetic		11	7
Toluidine	95534	XU2975000	Indirect		30	3
Vinyl Chloride	75014	KU9625000	Indirect		31	18
					31	

[a]American Chemical Society's classification number.

[b]Registry of Toxic Effects of Chemical Substances classification number.

APPENDIX B

CHARACTERISTICS OF THE TUMORIGENICITY AND MUTAGENICITY MASTER FILES

CHARACTERISTICS OF THE TUMORIGENICITY "MASTER FILE"

Routes of Administration

Intracerebral (ice)	Administration into the cerebrum
Intracervical (icv)	Administration into the cervix
Inhalation (ihl)	Inhalation in chamber, by cannulation, or through mask
Implant (imp)	Placed surgically within body
Intramuscular (ims)	Administration into the muscle by hypodermic needle
Intrapleural (ipl)	Administration into the pleural cavity by hypodermic needle
Intraperitoneal (ipr)	Administration into the peritoneal cavity
Intrarenal (irn)	Administration into the kidney
Intratracheal (itr)	Administration into the trachea
Intratesticular (itt)	Administration into the testes
Intravaginal (ivg)	Administration into the vagina
Intravenous (ivn)	Administration directly into the vein by hypodermic needle
Multiple (mul)	Administration by more than one route to single animals
Ocular (ocu)	Administration directly onto the surface of the eye, or into the conjunctival sac
Oral (orl)	Per os, intragastric, feeding, or introduction with drinking water
Parenteral (par)	Administration into the body through the skin
Rectal (rec)	Administration into the rectum or colon in the form of enema or suppository
Subcutaneous (scu)	Administration under the skin
Skin (skn)	Application directly onto the skin, either intact or abraded.

Test Species

Cat (cat)	Hamster (ham)
Chicken (ckn)	Monkey (mky)
Duck (dck)	Mouse (mus)
Dog (dog)	Pig (pig)
Frog (frg)	Rat (rat)
Guinea Pig (gpg)	Rabbit (rbt)
Gerbil (gbl)	

Units of Dose

g/kg	ppb
mg/kg	ppm
mg/m^3	µg/kg
ng/kg	$\mu g/m^3$
ng/m^3	

APPENDIX C

EXAMPLE OF THE MASTER FILE OF TUMIGENICITY DATA

EXAMPLE OF TUMORIGENICITY MASTER FILE

NOTE- This appendix is a sample abstracted from the Master File containing data on tumorigenicity experiments obtained from the RTECS data base. There are 658 experiments listed in the original file. Data from seven compounds are displayed in this appendix. Explanations for the abbreviations are found in Appendix B. Data in the file included the Registry of Toxic Effects of Chemical Substances classification number (RTECS), the American Chemical Society's classification number (CAS), the molecular weight of the compound (MW), the type of dose as recorded by the Registry (INDI), the test species exposed to the agent (SPE), the route of the exposure (RTE), the cumulative dose administered in the experiment (DOSE), and the units of the exposure (UNIT). A space has been inserted between the results for the different chemicals. Please observe the variation in the data between the different compounds. The mutagenicity data for these compounds is seen in Appendix D.

RTECS	CAS	MW	INDI	SPE	RTE	DOSE	UNIT
AC8925000	62555	75	TDLo	rat	orl	1008.00	mg/kg
AC8925000	62555	75	TDLo	mus	orl	10.00	gm/kg
AC8925000	62555	75	TD	rat	orl	6000.00	mg/kg
AC8925000	62555	75	TD	rat	orl	7200.00	mg/kg
AC8925000	62555	75	TD	rat	orl	9000.00	mg/kg
AC8925000	62555	75	TD	rat	orl	9900.00	mg/kg
AC8925000	62555	75	TD	rat	orl	1600.00	mg/kg
AC8925000	62555	75	TD	rat	orl	5140.00	mg/kg
AC8925000	62555	75	TD	rat	orl	7665.00	mg/kg
AC8925000	62555	75	TD	mus	orl	7956.00	mg/kg
AC8925000	62555	75	TD	rat	orl	4320.00	mg/kg
AC8925000	62555	75	TD	mus	orl	18360.00	mg/kg
AM4375000	62442	179	TDLo	rat	orl	572.00	gm/kg
AM4375000	62442	179	TDLo	mus	orl	1008.00	gm/kg
AM4375000	62442	179	TD	mus	orl	484.00	gm/kg
AM4375000	62442	179	TD	rat	orl	9450.00	mg/kg
AM4375000	62442	179	TD	rat	orl	206.00	gm/kg
AS3500000	3688537	248	TDLo	rat	orl	52.00	gm/kg
AS3500000	3688537	248	TDLo	mus	orl	156.00	gm/kg
AS3500000	3688537	248	TDLo	mus	scu	150.00	mg/kg
AS3500000	3688537	248	TDLo	ham	orl	63.00	gm/kg
AS3500000	3688537	248	TD	rat	orl	25.00	gm/kg
AS3500000	3688537	248	TD	mus	orl	211.00	gm/kg
AS3500000	3688537	248	TD	mus	orl	158.00	gm/kg
AS3500000	3688537	248	TD	mus	orl	42.00	gm/kg
AS3500000	3688537	248	TD	ham	orl	127.00	gm/kg
AS3500000	3688537	248	TD	ham	orl	116.00	gm/kg
AS3500000	3688537	248	TD	mus	orl	32400.00	mg/kg

Example of Tumorigenicity Master File (cont.)

RTECS	CAS	MW	INDI	SPE	RTE	DOSE	UNIT
AT5250000	107131	53	TDLo	rat	orl	18200.00	mg/kg
AT5250000	107131	53	TCLo	rat	ihl	5.00	ppm
AT5250000	107131	53	TC	rat	ihl	20.00	ppm
AT5250000	107131	53	TC	rat	ihl	40.00	ppm
AT5250000	107131	53	TD	rat	orl	3640.00	mg/kg
CY1400000	71432	78	TDLo	rat	orl	52.00	gm/kg
CY1400000	71432	78	TCLo	rat	ihl	1200.00	ppm
CY1400000	71432	78	TDLo	mus	orl	18250.00	mg/kg
CY1400000	71432	78	TCLo	mus	ihl	300.00	ppm
CY1400000	71432	78	TDLo	mus	skn	1200.00	gm/kg
CY1400000	71432	78	TDLo	mus	ipr	1200.00	mg/kg
CY1400000	71432	78	TDLo	mus	scu	600.00	mg/kg
CY1400000	71432	78	TDLo	mus	par	670.00	mg/kg
CY1400000	71432	78	TD	rat	orl	52.00	gm/kg
CY1400000	71432	78	TD	rat	orl	10.00	gm/kg
CY1400000	71432	78	TC	mus	ihl	1200.00	ppm
CY1400000	71432	78	TD	mus	orl	2400.00	mg/kg
DC9625000	92875	184	TDLo	rat	orl	108.00	mg/kg
DC9625000	92875	184	TCLo	rat	ihl	10.00	mg/m3
DC9625000	92875	184	TDLo	rat	scu	2025.00	mg/kg
DC9625000	92875	184	TDLo	rat	itr	315.00	mg/kg
DC9625000	92875	184	TDLo	mus	scu	8400.00	mg/kg
DC9625000	92875	184	TDLo	ham	orl	75.00	mg/kg
DC9625000	92875	184	TD	mus	scu	1620.00	gm/kg
DC9625000	92875	184	TD	rat	scu	850.00	mg/kg
DC9625000	92875	184	TD	rat	scu	800.00	mg/kg
DD0525000	91941	253	TDLo	rat	orl	17.00	gm/kg
DD0525000	91941	253	TDLo	rat	scu	7.00	gm/kg
DD0525000	91941	253	TDLo	mus	orl	5100.00	mg/kg
DD0525000	91941	253	TDLo	mus	scu	320.00	mg/kg
DD0525000	91941	253	TDLo	mus	scu	5200.00	mg/kg
DD0525000	91941	253	TDLo	dog	orl	17.00	gm/kg
DD0525000	91941	253	TDLo	ham	orl	176.00	gm/kg
DD0525000	91941	253	TD	rat	orl	20.00	gm/kg
DD0525000	91941	253	TD	rat	orl	21.00	gm/kg

APPENDIX D

EXAMPLE OF THE MASTER FILE OF MUTAGENICITY DATA

EXAMPLE OF MUTAGENICITY MASTER FILE

NOTE- This appendix is a sample abstracted from the Master File containing data on mutagenicity experiments obtained from the RTECS data base. There are 2140 experiments listed in this file. Explanations for the abbreviations are found in Appendix B. The data used for this analysis includes the Registry of Toxic Eeffects of Chemical Substances classification number (RTECS), the American Chemical Society's classification number (CAS), the molecular weight of the compound (MW), the test species (or source of cells for the experiment) exposed to the agent (SPE), the endpoint measured (END), the type of cells used or the route of exposure (CTR), the cumulative dose administered in the experiment (DOSE), and the units of the exposure (UNIT). A space has been inserted between the results for the different chemicals. Please observe the variation in the data between the different compounds. The tumorgenicity data on these seven compounds is seen in Appendix C.

RTECS	CAS	MW	SPE	END	CTR	DOSE	UNIT
AC8925000	62555	75	esc	dnd		50	umol/L
AC8925000	62555	75	smc	mmo		19900	umol/L
AC8925000	62555	75	esc	mrc		400	ug/well
AC8925000	62555	75	ham	otr	emb	100	ug/L
AC8925000	62555	75	dmg	sln	par	2500	ppm
AC8925000	62555	75	dmg	sln	orl	100	ppm
AC8925000	62555	75	rat	dnd	lvr	300	mmol/L
AC8925000	62555	75	smc	mrc		3000	ppm
AC8925000	62555	75	rat	dns	orl	2940	mg/kg
AC8925000	62555	75	mky	mnt	kdy	100	mg/L
AC8925000	62555	75	rat	otr	emb	30	mg/L
AC8925000	62555	75	mky	cyt	kdy	50	mg/L
AC8925000	62555	75	hmn	cyt	fbr	1	gm/L
AC8925000	62555	75	rat	cyt	par	150	mg/kg
AC8925000	62555	75	rat	oms	ipr	5	mg/kg
AC8925000	62555	75	mus	hma	sat	125	mg/kg
AC8925000	62555	75	rat	dnd	ipr	60	mg/kg
AC8925000	62555	75	rat	cyt	ipr	150	mg/kg
AM4375000	62442	179	sat	mma		333	ug/plate
AM4375000	62442	179	rat	hma	sat	200	ug/kg
AM4375000	62442	179	rat	dnd	orl	82500	ug/kg
AM4375000	62442	179	ham	mma	lng	1	mmol/L
AM4375000	62442	179	esc	dnr		2	mg/well
AM4375000	62442	179	ham	sce	lng	100	mg/L
AM4375000	62442	179	mus	dni	oth	50	mg/L
AM4375000	62442	179	ham	cyt	fbr	800	mg/L
AM4375000	62442	179	ham	cyt	lng	800	mg/L

Example of Mutagenicity Master File (Cont.)

RTECS	CAS	MW	SPE	END	CTR	DOSE	UNIT
AM4375000	62442	179	mus	sce	ipr	165	mg/kg
AM4375000	62442	179	rat	dnd	ipr	165	mg/kg
AM4375000	62442	179	rat	bfa	sat	800	mg/kg
AM4375000	62442	179	ham	bfa	sat	1600	mg/kg
AM4375000	62442	179	mus	dni	ipr	20	gm/kg
AM4375000	62442	179	mus	dnd	ipr	400	mg/kg
CY1400000	71432	78	dmg	slt	orl	11250	umol/L
CY1400000	71432	78	hmn	sce	lym	200	umol/L
CY1400000	71432	78	hmn	dni	hla	2200	umol/L
CY1400000	71432	78	hmn	oms	lym	5	umol/L
CY1400000	71432	78	hmn	dni	leu	2200	umol/L
CY1400000	71432	78	ham	sln	lvr	62500	ug/L
CY1400000	71432	78	rat	dni	ihl	400	ppm
CY1400000	71432	78	mus	otr	fbr	100	ug/L
CY1400000	71432	78	mus	msc	lym	12500	ug/L
CY1400000	71432	78	hmn	cyt	leu	1	mmol/L
CY1400000	71432	78	mus	cyt	ihl	3000	ppm
CY1400000	71432	78	mus	mnt	ihl	10	ppm
CY1400000	71432	78	mus	mma	lym	62500	ug/L
CY1400000	71432	78	hmn	cyt	unr	10	ppm
CY1400000	71432	78	rat	dnd	orl	100	nmol/kg
CY1400000	71432	78	mus	mnt	ipr	264	ug/kg
CY1400000	71432	78	rat	sce	ihl	3	ppm
CY1400000	71432	78	man	cyt	ihl	125	ppm
CY1400000	71432	78	mus	sce	ihl	10	ppm
CY1400000	71432	78	rat	mnt	ihl	1	ppm
CY1400000	71432	78	asn	sln		35000	ppm
CY1400000	71432	78	ham	otr	emb	100	ug/L
CY1400000	71432	78	dmg	slt	ihl	27000	ppm
CY1400000	71432	78	rat	dns	lvr	1	mmol/L
CY1400000	71432	78	rat	cyt	ihl	300	mg/m3
CY1400000	71432	78	smc	mrc		275	mg/L
CY1400000	71432	78	rat	oms	bmr	1	mmol/L
CY1400000	71432	78	mus	oms	lym	10	mmol/L
CY1400000	71432	78	smc	mmo		275	mg/L
CY1400000	71432	78	ham	dnd	ovr	17	mmol/L
CY1400000	71432	78	cat	oms	bmr	1	mmol/L
CY1400000	71432	78	mus	mma	emb	2500	mg/L
CY1400000	71432	78	grh	oms	ihl	1053	mg/L
CY1400000	71432	78	hmn	cyt	lym	1	mg/L

Example of Mutagenicity Master File (Cont.)

RTECS	CAS	MW	SPE	END	CTR	DOSE	UNIT
CY1400000	71432	78	smc	mma		549	mg/L
CY1400000	71432	78	smc	cyt		12	mmol/L
CY1400000	71432	78	rbt	oms	bmr	1	mmol/L
CY1400000	71432	78	ham	cyt	ovr	600	mg/L
CY1400000	71432	78	ham	cyt	lng	550	mg/L
CY1400000	71432	78	rat	oms	lvr	1	mmol/L
CY1400000	71432	78	mus	mnt	orl	40	mg/kg
CY1400000	71432	78	mus	mnt	scu	440	mg/kg
CY1400000	71432	78	rat	oms	scu	2200	mg/kg
CY1400000	71432	78	rat	oms	scu	1	gm/L
CY1400000	71432	78	rat	cyt	scu	12	gm/kg
CY1400000	71432	78	mus	sce	ipr	5	gm/kg
CY1400000	71432	78	mus	dlt	ipr	5	mg/kg
CY1400000	71432	78	mus	otr	emb	1	gm/L
CY1400000	71432	78	mus	dlt	orl	1	mg/kg
CY1400000	71432	78	mus	dni	orl	20	gm/kg
CY1400000	71432	78	rbt	cyt	scu	8400	mg/kg
CY1400000	71432	78	rbt	dni	scu	2	gm/kg
CY1400000	71432	78	mus	cyt	orl	20	mg/kg
CY1400000	71432	78	mus	cyt	ipr	100	mg/kg
CY1400000	71432	78	nml	oms	ipr	75	gm/kg
DD0525000	91941	253	sat	mma		5	ug/plate
DD0525000	91941	253	sat	mmo		50	ug/plate
DD0525000	91941	253	ham	otr	kdy	80	ug/L
DD0525000	91941	253	hmn	dns	hla	100	nmol/L
DD0525000	91941	253	mam	dnd	lym	25500	nmol/L
DD0525000	91941	253	rat	bfa	sat	40	mg/kg
DC9625000	92875	184	man	sce	ihl	7	ug/m3
DC9625000	92875	184	rbt	dns	lvr	1	umol/L
DC9625000	92875	184	esc	mma		100	ug/plate
DC9625000	92875	184	sat	mma		25	ug/plate
DC9625000	92875	184	esc	mrc		150	ug/well
DC9625000	92875	184	dog	dnd	orl	60	umol/kg
DC9625000	92875	184	ham	dnd	lng	100	umol/L
DC9625000	92875	184	omi	oms		30	umol/L
DC9625000	92875	184	hmn	dns	hla	100	umol/L
DC9625000	92875	184	mus	dnr	lvr	60	umol/L
DC9625000	92875	184	dog	oms	oth	100	umol/L
DC9625000	92875	184	rat	bfa	sat	250	umol/kg

Example of Mutagenicity Master File (Cont.)

RTECS	CAS	MW	SPE	END	CTR	DOSE	UNIT
DC9625000	92875	184	dog	dnd	oth	100	umol/L
DC9625000	92875	184	hmn	dni	hla	600	umol/L
DC9625000	92875	184	sat	nmo		25	ug/plate
DC9625000	92875	184	mam	dnd	lym	30	umol/L
DC9625000	92875	184	rat	dns	lvr	100	nmol/L
DC9625000	92875	184	hmn	dnd	fbr	3	mmol/L
DC9625000	92875	184	rat	dnd	lvr	3	mmol/L
DC9625000	92875	184	ham	dns	lvr	20	nmol/L
DC9625000	92875	184	ham	otr	kdy	80	ug/L
DC9625000	92875	184	ham	otr	emb	50	ug/L
DC9625000	92875	184	mus	dnd	orl	9600	ug/kg
DC9625000	2875	184	rat	cyt	lvr	12500	ug/L
DC9625000	92875	184	rat	dnd	par	11600	ug/kg
DC9625000	92875	184	hmn	dns	fbr	160	ug/L
DC9625000	92875	184	mus	otr	emb	2500	ug/L
DC9625000	92875	184	mus	msc	lym	500	ug/L
DC9625000	92875	184	mus	sce	ipr	7700	ug/kg
DC9625000	92875	184	mus	mnt	ipr	6400	ug/kg
DC9625000	92875	184	rat	mnt	scu	410	mg/kg
DC9625000	92875	184	mus	dni	orl	200	mg/kg
DC9625000	92875	184	mus	dnd	ipr	150	mg/kg
DC9625000	92875	184	smc	mrc		100	mg/L
DC9625000	92875	184	ham	slt	ovr	600	mg/L
DC9625000	92875	184	ham	cyt	ovr	1670	mg/L
DC9625000	92875	184	ham	sce	ovr	3330	mg/L
DC9625000	92875	184	esc	dnr		1	mg/plate
DC9625000	92875	184	rat	dnd	orl	200	mg/kg
DC9625000	92875	184	rat	dns	orl	200	mg/kg
DC9625000	92875	184	rat	dnd	ipr	63	mg/kg
DC9625000	92875	184	dmg	sln	par	5	mmol/L
DC9625000	92875	184	rat	hma	s[illegible]	1	mmol/kg
DC9625000	92875	184	hmn	sce	lym	23	mg/L
DC9625000	92875	184	esc	pic		100	mmol/L
DC9625000	92875	184	smc	dnr		100	mg/L
DC9625000	92875	184	smc	sln		50	mg/L
DC9625000	92375	184	ham	msc	ovr	20	mg/L
DC9625000	92875	184	rat	dns	orl	200	mg/kg
DC9625000	92875	184	ofs	cyt	ipr	10	mg/kg
DC9625000	92875	184	mus	dni	orl	20	gm/kg
DC9625000	92875	184	ofs	cyt	ipr	10	mg/kg
DC9625000	92875	184	bcs	dnd		2	mg/disc

Example of Mutagenicity Master File (Cont.)

RTECS	CAS	MW	SPE	END	CTR	DOSE	UNIT
AT5250000	107131	53	smc	mma		800	ug/L
AT5250000	107131	53	rat	dnd	lvr	16500	umol/L
AT5250000	107131	53	dmg	slt	orl	1520	umol/L
AT5250000	107131	53	rat	oms	lvr	16500	umol/L
AT5250000	107131	53	smc	mmo		800	ug/L
AT5250000	107131	53	rat	oms	orl	46500	ug/kg
AT5250000	107131	53	rat	dnd	orl	46500	ug/kg
AT5250000	107131	53	smc	oms		500	ppm
AT5250000	107131	53	ham	cyt	lvr	2500	ug/L
AT5250000	107131	53	ham	cyt	lng	6250	ug/L
AT5250000	107131	53	mus	otr	emb	8800	ug/L
AT5250000	107131	53	ham	cyt	ovr	4	mmol/L
AT5250000	107131	53	ham	sce	ovr	2	mmol/L
AT5250000	107131	53	ham	dnd	ovr	3710	mg/L
AT5250000	107131	53	ham	dnd	emb	200	mg/L
AT5250000	107131	53	rat	dns	lvr	1	mmol/L
AT5250000	107131	53	asn	sln		800	mg/L
AT5250000	107131	53	asn	mrc		806	mg/L
AT5250000	107131	53	mus	mma	emb	50	mg/L
AT5250000	107131	53	smc	mrc		14	mg/L
AT5250000	107131	53	mus	mma	lym	161	mg/L
AT5250000	107131	53	mam	dnd	lym	8	mmol/L
AT5250000	107131	53	ham	mnt	ovr	100	mmol/L
AT5250000	107131	53	ham	otr	emb	2	mg/L
AT5250000	107131	53	rat	bfa	sat	30	mg/kg
AT5250000	107131	53	mus	bfa	sat	30	mg/kg
AS3500000	3688537	248	ham	dni	lng	10	umol/L
AS3500000	3688537	248	smc	mrc		330	umol/L
AS3500000	3688537	248	mus	msc	mmr	1	umol/L
AS3500000	3688537	248	ham	sce	ovr	160	ug/L
AS3500000	3688537	248	hmn	cyt	lym	30	umol/L
AS3500000	3688537	248	smc	mmo		330	umol/L
AS3500000	3688537	248	ham	msc	emb	100	umol/L
AS3500000	3688537	243	nsc	mmo		30	umol/L
AS3500000	3688537	248	hmn	sce	lym	500	ug/L
AS3500000	3688537	248	mus	cyt	mmr	10	umol/L
AS3500000	3688537	248	ham	otr	emb	5	umol/L
AS3500000	3688537	248	esc	dnd		2	umol/L
AS3500000	3688537	248	sat	mmo		4	ug/L

Example of Mutagenicity Master File (Cont.)

RTECS	CAS	MW	SPE	END	CTR	DOSE	UNIT
AS3500000	3688537	248	dmg	sln	orl	500	umol/L
AS3500000	3688537	248	hmn	dns	fbr	4	umol/L
AS3500000	3688537	248	esc	mrc		500	nmol/well
AS3500000	3688537	248	sat	dnr		500	nmol/well
AS3500000	3688537	248	ham	msc	lng	100	nmol/L
AS3500000	3688537	248	bcs	mmo		100	nmol/L
AS3500000	3688537	248	esc	dnr		700	nmol/L
AS3500000	3688537	248	sat	mma		20	ng/plate
AS3500000	3688537	248	esc	mmo		100	ng/plate
AS3500000	3688537	248	bcs	dnr		50	ppm
AS3500000	3688537	248	esc	pic		40	ug/L
AS3500000	3688537	248	ham	cyt	ovr	500	ug/L
AS3500000	3688537	248	sat	pic		3	pmol/plate
AS3500000	3688537	248	dmg	slt	orl	50	ppm
AS3500000	3688537	248	esc	mma		25	mg/L
AS3500000	3688537	248	hmn	cyt	emb	3	mg/L
AS3500000	3688537	248	ham	cyt	fbr	10	mg/L
AS3500000	3688537	248	ofs	spm	mul	5	mg/L
AS3500000	3688537	248	rat	bfa	sat	1200	mg/kg
AS3500000	3688537	248	rat	mnt	ipr	60	mg/kg
AS3500000	3688537	248	omi	mrc		25	mg/L
AS3500000	3688537	248	mus	dnd	mmr	1	mmol/L
AS3500000	3688537	248	rat	dns	oth	10	mmol/L
AS3500000	3688537	248	omi	mmo		25	mg/L
AS3500000	3688537	248	ham	cyt	lng	6	mg/L
AS3500000	3688537	248	eug	mmo		50	mg/L
AS3500000	3688537	248	ham	otr	ipr	20	mg/kg
AS3500000	3688537	248	rat	cyt	par	300	mg/kg
AS3500000	3688537	248	ham	cyt	ipr	20	mg/kg
AS3500000	3688537	248	ham	msc	ipr	20	mg/kg
AS3500000	3688537	248	ham	cyt	unr	20	mg/kg
AS3500000	3688537	248	rat	cyt	ipr	80	mg/kg
AS3500000	3688537	248	ham	hma	fbr	50	mg/kg

APPENDIX E

EXAMPLES OF ARRAYS OF TUMORIGENIC RELATIVE POTENCIES

NOTE- This appendix illustrates the differences in the arrays of test compounds standardized to different reference compounds. The means of summarizing the array (median and interquartile range) is also demonstrated. The compounds are identified by their CAS numbers (TESTCAS). The criteria used to match the tumorigenicity experiments included the route of administration (RTE), the endpoint of the experiment (INDI), the species (SPE). The relative potencies of the test compounds standardized to the reference compounds is seen in the last column (RELPOT). This example includes data from three compounds (cyclophosphamide, estradiol, and dichlorobenzidine) standardized to two reference compounds (dimethylnitrosamine and benzo(a)pyrene). Notice the differences in the arrays. Mutagenicity data on the same test and reference compounds is displayed in Appendix E.

TUMORIGENIC RELATIVE POTENCIES

REFERENCE COMPOUND = BENZO(a)PYRENE

TEST COMPOUND = CYCLOPHOSPHAMIDE

TESTCAS	RTE	INDI	SPE	RELPOT
50180	scu	TD	mus	0.0023016
50180	scu	TD	mus	0.0034524
50180	ipr	TDLo	mus	0.0053113
50180	scu	TDLo	mus	0.0068946
50180	orl	TDLo	rat	0.0327068
50180	ivn	TDLo	rat	0.0597527
50180	ipr	TDLo	mus	0.1593407
50180	scu	TDLo	mus	0.3677092

N = 8
MEDIAN = .0198
INTERQUARTILE RANGE = .0044 - .1095 (24.896)

REFERENCE COMPOUND = BENZO(a)PYRENE

TEST COMPOUND = ESTRADIOL

TESTCAS	RTE	INDI	SPE	RELPOT
50282	imp	TDLo	rat	0.0004626
50282	imp	TD	rat	0.0086348
50282	ipr	TDLo	rat	0.0123356
50282	orl	TDLo	mus	13.0268197
50282	imp	TDLo	mus	2159.0065290

N = 5
MEDIAN = .0123
INTERQUARTILE RANGE = .00454 - 1084.516 (238880.324)

REFERENCE COMPOUND = BENZO(a)PYRENE

TEST COMPOUND = DICHLOROBENZIDINE

TESTCAS	RTE	INDI	SPE	RELPOT
91941	scu	TDLo	rat	0.0000653
91941	orl	TDLo	rat	0.0008859
91941	scu	TDLo	mus	0.0017376
91941	orl	TDLo	ham	0.0023958
91941	scu	TDLo	mus	0.0282366
91941	scu	TDLo	mus	0.0926740
91941	orl	TDLo	mus	0.1377996
91941	scu	TDLo	mus	1.5059524

N = 8
MEDIAN = .0153
INTERQUARTILE RANGE = .0013 - .1152 (88.643)

REFERENCE COMPOUND = DIMETHYLNITRSOAMINE

TEST COMPOUND = CYCLOPHOSPHAMIDE

TESTCAS	RTE	INDI	SPE	RELPOT
50180	ipr	TDLo	mus	0.0126611
50180	ivn	TDLo	rat	0.0939149
50180	scu	TDLo	mus	0.0991324
50180	orl	TD	rat	0.1166418
50180	orl	TD	rat	0.1378001
50180	orl	TDLo	rat	0.1707824
50180	scu	TDLo	mus	0.1826123
50180	orl	TD	rat	0.2122280
50180	orl	TDLo	rat	0.2227596
50180	orl	TD	rat	0.3165993
50180	orl	TD	rat	0.3740289
50180	orl	TD	rat	0.5760474
50180	orl	TD	rat	0.9331347
50180	orl	TD	rat	1.1024010
50180	orl	TD	rat	1.6978239

N = 15
MEDIAN = .2122
INTERQUARTILE RANGE = .1166 - .5760 (4.9399)

REFERENCE COMPOUND = DIMETHYLNITRSOAMINE

TEST COMPOUND = ESTRADIOL

TESTCAS	RTE	INDI	SPE	RELPOT
50282	orl	TDLo	mus	0.0088724
50282	ipr	TDLo	rat	0.0787645
50282	orl	TD	mus	0.9189187
50282	orl	TDLo	mus	5.0698974
50282	orl	TD	mus	30.9090920

N = 5
MEDIAN = .9189
INTERQUARTILE RANGE = .0438 - 17.9894 (410.718)

REFERENCE COMPOUND = DIMETHYLNITRSOAMINE

TEST COMPOUND = DICHLOROBENZIDINE

TESTCAS	RTE	INDI	SPE	RELPOT
91941	orl	TDLo	mus	0.0000939
91941	orl	TDLo	ham	0.0002525
91941	orl	TDLo	rat	0.0046256
91941	orl	TDLo	rat	0.0060334
91941	orl	TD	rat	0.0068378
91941	orl	TD	rat	0.0071797
91941	scu	TDLo	rat	0.0119496
91941	orl	TD	rat	0.0185598
91941	orl	TD	rat	0.0194878
91941	scu	TDLo	mus	0.0249844
91941	scu	TDLo	mus	0.0460239
91941	orl	TDLo	mus	0.0536301
91941	orl	TD	rat	0.0547027
91941	orl	TD	rat	0.0574378
91941	scu	TDLo	mus	0.4059966
91941	scu	TDLo	mus	0.7478885

N = 16
MEDIAN = .019
INTERQUARTILE RANGE = .0064 - .054 (8.463)

APPENDIX F

EXAMPLES OF ARRAYS MUTAGENIC RELATIVE POTENCIES

EXAMPLES OF ARRAYS MUTAGENIC RELATIVE POTENCIES

NOTE- This appendix illustrates the differences in the arrays of test compounds standardized to different reference compounds. The means of summarizing the array (median and interquartile range) is also demonstrated. The compounds are identified by their CAS number (TESTCAS). The criteria used to match the mutagenic test systems included the cell type or route of administration (CTR), the endpoint (IND), the species used in the experiment (SPE). The relative potencies of the test compounds standardized to the reference compounds is seen in the last column (RELPOT).

This example includes data from three compounds (cyclophosphamide, estradiol, and dichlorobenzidine) standardized to two reference compounds (Dimethylnitrosamine and benzo(a)pyrene). Notice the differences in the arrays. Tumorigenicity data on the same test and reference compounds is displayed in Appendix F.

REFERENCE COMPOUND = BENZO(a)PYRENE

TEST COMPOUND = CYCLOPHOSPHAMIDE

TESTCAS	CTR	IND	SPE	RELPOT
50180		mma	esc	0.0000200
50180		mrc	smc	0.0000794
50180	fbr	cyt	ham	0.0003968
50180	lvr	sce	rat	0.0004176
50180	lvr	sln	ham	0.0005179
50180	lng	mma	ham	0.0006525
50180	leu	cyt	hmn	0.0007937
50180	lng	sce	ham	0.0016704
50180		pic	esc	0.0041765
50180		mma	smc	0.0074312
50180	emb	otr	ham	0.0082857
50180	kdy	sce	mky	0.0100000
50180	orl	sln	dmg	0.0100000
50180	lvr	dns	rat	0.0100000
50180	emb	otr	mus	0.0103565
50180	hla	dni	hmn	0.0116667
50180	emb	sce	mus	0.0261000
50180	lym	msc	mus	0.0582588
50180	ovr	sce	ham	0.1000000
50180		dnd	bcs	0.1035783
50180	orl	dns	mus	0.1242856
50180	ipr	spm	ham	0.1294642
50180	fbr	sce	ham	0.1666667
50180		dnr	esc	0.1984127

Examples of Arrays Mutagenic Relative Potencies

TESTCAS	CTR	IND	SPE	RELPOT
50180	sat	bfa	rat	0.2071426
50180	oth	sce	ham	0.2583898
50180	orl	cyt	mus	0.2589284
50180	par	sln	dmg	0.2604167
50180		mma	sat	0.3157895
50180	fbr	sce	hmn	0.3968300
50180	ipr	spm	mus	0.4142857
50180	ipr	dni	mus	0.6904762
50180	ipr	dnd	mus	0.6904762
50180	kdy	otr	ham	1.0358891
50180		sln	smc	1.5535713
50180	oth	sce	rat	1.6666667
50180	lym	sce	rbt	2.0000000
50180	sat	bfa	mus	2.0714284
50180	lym	mma	mus	2.0714360
50180	ovr	cyt	ham	2.6099997
50180	emb	otr	rat	3.1899974
50180	unr	mnt	mus	3.8839255
50180	lng	cyt	ham	3.9682540
50180	par	sce	ckn	4.0065147
50180	unr	spm	mus	4.1428568
50180	lym	sce	hmn	5.0000000
50180	scu	mnt	mus	5.1785683
50180	ipr	mnt	mus	6.2142809
50180	ipr	dlt	mus	7.7678564
50180	ipr	dnd	rat	8.2857113
50180	unr	sce	mus	8.2857179
50180	ipr	sce	mus	8.6309127
50180	ivn	cyt	rat	15.6926137
50180	ovr	cyt	ham	17.2964268
50180	unr	sce	ham	25.8929634
50180	unr	cyt	rat	48.6785122
50180	ipr	cyt	ham	62.7704549
50180	ipr	dni	mus	138.0952487
50180	lym	cyt	hmn	248.6631854
50180	ipr	sce	ham	932.1471525
50180	hla	dns	hmn	10000.0000000

N = 61
MEDIAN = .414
INTERQUARTILE RANGE = .011 - 5.137 (467.00)

Examples of Arrays Mutagenic Relative Potencies

REFERENCE COMPOUND = BENZO(a)PYRENE

TEST COMPOUND = ESTRADIOL

TESTCAS	CTR	IND	SPE	RELPOT
50282	oth	dnd	rat	1.5900000
50282	lvr	dns	rat	5.0000000
50282	ovr	cyt	ham	20.0000000
50282	oth	dnd	rat	30.0000000
50282	oth	dnd	rat	100.0000000
50282	ovr	cyt	ham	132.5396820
50282	oth	dnd	rat	150.0000000
50282	oth	dnd	rat	3100.0000000
50282	lym	dnd	mam	6000.0000000

N = 9
MEDIAN = 100
INTERQUARTILE RANGE = 12.50 - 1625.00 (130)

REFERENCE COMPOUND = BENZO(a)PYRENE

TEST COMPOUND = DICHLOROBENZIDINE

TESTCAS	CTR	IND	SPE	RELPOT
91941		mmo	sat	0.0399798
91941		mma	sat	0.0606061
91941	sat	bfa	rat	0.2509918
91941	kdy	otr	ham	1.0041113
91941	lym	dnd	mam	1.1764706
91941	hla	dns	hmn	10000.0000000

N = 6
MEDIAN = .6276
INTERQUARTILE RANGE = .050 - 5000.588 (99415.273)

Examples of Arrays Mutagenic Relative Potencies

REFERENCE COMPOUND = DIMETHYLNITROSAMINE

TEST COMPOUND = CYCLOPHOSPHAMIDE

TESTCAS	CTR	IND	SPE	RELPOT
50180		mma	esc	0.0000140
50180	ipr	dnd	ham	0.0017631
50180	emb	otr	ham	0.0070543
50180	smc	hma	mus	0.0116000
50180	hla	cyt	hmn	0.0124286
50180	ipr	dnd	mus	0.0268054
50180	ipr	spm	mus	0.0282162
50180	orl	dns	mus	0.0423243
50180	srm	hma	mus	0.0486487
50180	ipr	dns	mus	0.0529054
50180	ovr	sce	ham	0.0675680
50180	sat	hma	mus	0.0981081
50180	par	sln	dmg	0.1041667
50180	ipr	dns	rat	0.1410810
50180	ipr	dlt	mus	0.1551893
50180	ipr	sce	mus	0.2351339
50180	orl	sln	dmg	0.2500000
50180	lvr	dns	rat	0.8108108
50180	orl	mnt	mus	0.8817566
50180	unr	sln	dmg	1.2500000
50180	orl	dns	rat	1.4108110
50180	lym	mma	mus	1.7635329
50180	lng	mma	ham	2.6099984
50180	ipr	mnt	ham	3.5270239
50180	kdy	otr	ham	3.5272431
50180	fbr	cyt	ham	4.0000000
50180	lng	sce	ham	4.1760628
50180	smc	hma	rat	5.0000000
50180	ipr	slt	mus	5.2199968
50180	ipr	dnd	rat	7.0540478
50180	ipr	mnt	mus	9.8756701
50180		mrc	smc	10.0000000
50180		mma	smc	13.1086142
50180	fbr	sce	ham	13.3333333
50180	ipr	cyt	rat	21.3758868
50180	orl	sce	ham	35.2702497
50180	lym	sce	hmn	39.1891900

REFERENCE COMPOUND = DIMETHYLNITROSAMINE

TEST COMPOUND = CYCLOPHOSPHAMIDE (continued)

TESTCAS	CTR	IND	SPE	RELPOT
50180	ipr	sce	ham	52.9056481
50180	lng	cyt	ham	67.5675680
50180	ovr	cyt	ham	70.4699931
50180		dnr	bcs	88.1756762
50180	lym	msc	mus	163.1250053
50180	hla	dni	hmn	166.6666667
50180	fbr	sce	hmn	300.0000000
50180		mma	sat	355.6315789
50180	ipr	dni	mus	470.2703061
50180	emb	otr	rat	917.0264602
50180	hla	dns	hmn	1000.0000000
50180	oth	sce	ham	1043.9993530
50180	lvr	sce	rat	130500.6003000
50180	lym	cyt	hmn	1305483.0290000

N = 51
MEDIAN = 4.0
INTERQUARTILE RANGE = .104 - 67.568 (649.692)

REFERENCE COMPOUND = DIMETHYLNITROSAMINE

TEST COMPOUND = ESTRADIOL

TESTCAS	CTR	IND	SPE	RELPOT
50282	scu	dns	rat	0.3099492
50282	ipr	dns	rat	73.5228509
50282	lvr	dns	rat	405.4054000
50282	scu	dns	mus	463.3204571
50282	ovr	cyt	ham	540.0000000
50282	oth	dnd	rat	630.0000000
50282	oth	dnd	rat	17500.0000000
50282	lym	dnd	mam	86000.0000000
50282	oth	dnd	rat	100000.0000000

N = 9
MEDIAN = 540.00
INTERQUARTILE RANGE = 239.464 - 51750 (216.107)

REFERENCE COMPOUND = DIMETHYLNITROSAMINE

TEST COMPOUND = DICHLOROBENZIDINE

TESTCAS	CTR	IND	SPE	REL.POT
91941	kdy	otr	ham	3.4190386
91941	lym	dnd	mam	16.8627451
91941		mma	sat	68.2525253
91941	hla	dns	hmn	1000.0000000

N = 4
MEDIAN = 42.55
INTERQUARTILE RANGE = 3.419 - 1000 (292.483)

APPENDIX G

EXAMPLES OF REFERENCE-CHEMICAL-SPECIFIC-FILES

NOTE- These are examples of the tumorigenicity and mutagenicity reference-chemical-specific files. Each of these files has the following information; the name of all of the test chemicals (CHEMNAME), the number of bioassays which "matched" between the test compound and the reference compound (TOTALRECS), the median value of the array (MEDIAN), and the interquartile range of the array (RANGE). Data in this appendix are from the DMNA and B(a)P files.

TUMORGENICITY DATA STANDARDIZED TO DMNA

CHEMNAME	TOTALRECS	MEDIAN	RANGE
FORMALDEHYDE	0	0.0000000	0.00
METHYLIODIDE	0	0.0000000	0.00
ENDRIN	0	0.0000000	0.00
ANILINE	0	0.0000000	0.00
SACCHARINE	3	0.0003000	1030.00
BUTYLATEDHYDROXYANISOLE	21	0.0009000	14.34
PHENACETIN	12	0.0011700	66.70
BUTYLATEDHYDROXY-TOLUENE	9	0.0014000	480.00
CHLOROFORM	20	0.0029000	9.50
THIOUREA	15	0.0029200	19.10
CARBON TETRACHLORIDE	10	0.0030000	11.61
EPICHLOROHYDRIN	18	0.0038000	8.00
ACRYLAMIDE	18	0.0038000	22.00
BENZENE	18	0.0045000	14.48
VINYL CHLORIDE	10	0.0064000	18.50
ACRYLONITRILE	6	0.0107000	42.00
PHENOBARBITOL	5	0.0123000	18.00
HYDRAZINE	4	0.0126000	2200.00
ETHYLENE OXIDE	8	0.0141000	7.00
2-NAPTHYLAMINE	19	0.0157000	9.16
EDB	26	0.0171000	4.94
DBCP	9	0.0174700	0.03
DICHLOROBENZIDINE	16	0.0190000	8.40
DIMETHYLCARBAMOYL CHLORIDE	5	0.019600	0.01
THIOACETAMIDE	32	0.0198400	5.40
N-NITROSONORNICOTENE	15	0.0336000	4.80
PROPIOLACTONE	13	0.0363000	65.00
BENZIDINE	7	0.0497000	19.00
AZOTHIOPRINE	9	0.0581200	4.00
DDT	35	0.0673000	28.00
TOLUIDINE	4	0.0888000	69.00
CHLORDANE	4	0.1178000	1355.00
4-AMINOBIPHENYL	9	0.1202000	207.00
N-NITROSOPIPERIDINE	26	0.1269000	3.60
ETHYLENEIMINE	5	0.1978000	511.00

TUMORGENICITY DATA STANDARDIZED TO DMNA (Cont.)

CHEMNAME	TOTALRECS	MEDIAN	RANGE
CYCLOPHOSPHAMIDE	15	0.2122000	4.93
BCME	6	0.2214000	3.70
METHYLMETHANE SULFONATE	12	0.2382000	9.40
NICKEL SUBSULFIDE	5	0.6687000	7.40
DIMETHYLSULFATE	3	0.8332000	77.00
ESTRADIOL	5	0.9189000	410.00
N-METHYL-N-NITROSOUREA	21	0.9279000	7.30
CHORAMBUCIL	6	0.996800	26.50
ALDRIN	9	1.1020000	9.50
CIS-PLATIN	3	1.7513000	2.00
3-MC	26	2.2634000	38.80
DES	13	3.0644000	1444.78
RESERPINE	7	3.5052000	3.00
B(a)P	21	6.8108000	81.65
CADMIUM CHLORIDE	5	17.0891000	2.12
PHORBOL ACETATE	3	66.702500	1890.00
TCDD	24	6780.1566000	23.00

MUTAGENICITY DATA STANDARDIZED TO B(A)P

CHEMNAME	TOTALRECS	MEDIAN	RANGE
ENDRIN	0	0.00	0.00
BUTYLATEDHYDROXYANISOLE	0	0.00	0.00
B(a)P	0	0.00	0.00
VINYL CHLORIDE	14	0.0000020	21875.00
SACCHARINE	11	0.0012200	290.00
ACRYLONITRILE	18	0.0050000	1206.00
TOLUIDINE	25	0.0086000	161.00
FORMALDEHYDE	38	0.0099000	286.00
CHLOROFORM	7	0.0100000	57.00
ETHYLENE OXIDE	14	0.0100000	2262.00
HYDRAZINE	11	0.0198000	1649.00
METHYLMETHANE SULFONATE	104	0.0199000	640.00
ANILINE	8	0.0281000	116.00
DIMETHYLSULFATE	31	0.037500	1250.00
N-NITROSOPIPERIDINE	14	0.042500	33.00
PHENACETIN	14	0.0488000	1594.00
THIOACETAMIDE	11	0.0521000	66.00
PROPIOLACTONE	49	0.0571000	2374.00
ETHYLENEIMINE	16	0.0606000	2066.00
THIOUREA	8	0.0609000	931.00
DMNA	100	0.0642000	650.00
BENZENE	24	0.0852000	161.00
N-METHYL-N-NITROSOUREA	97	0.1220000	246.00
4-AMINOBIPHENYL	20	0.1461000	24.00
EDB	21	0.1984000	51.00
AZOTHIOPRINE	8	0.2015000	90.00
DIMETHYLCARBAMOYL CHLORIDE	18	0.2284000	451.00
ALDRIN	5	0.2667000	300.00
BCME	4	0.2723000	475.00
N-NITROSONORNICOTENE	3	0.2732000	840.00
2-NAPTHYLAMINE	39	0.2837000	29.00
CARBON TETRACHLORIDE	10	0.2971000	61.00
DBCP	8	0.2979000	257.00
NICKEL SUBSULFIDE	7	0.3174000	450.00
BENZIDINE	39	0.3408000	133.00
BHT (BUTYLATEDHYDROXY-TOLUENE)	3	0.3492000	280.00
DES	33	0.3545000	272.00
CYCLOPHOSPHAMIDE	61	0.4142000	463.00
EPICHLOROHYDRIN	21	0.4613000	1582.00
METHYLIODIDE	8	0.4649000	27378.00
PHENOBARBITOL	4	0.5060000	75000.00
DICHLOROBENZIDINE	6	0.6276000	22.00

MUTAGENICITY DATA STANDARDIZED TO B(A)P (continued)

CHEMNAME	TOTALRECS	MEDIAN	RANGE
3-MC	58	1.0000000	8.00
CHORAMBUCIL	15	1.2063000	3216.00
CADMIUM CHLORIDE	26	1.2460000	2588.00
DDT	11	1.4047000	19000.00
CHLORDANE	3	3.9680000	126.00
CIS-PLATIN	57	4.9762000	785.00
ACRYLAMIDE	27	9.8412000	124.00
TCDD	12	20.9324000	5490.00
PHORBOL ACETATE	18	27.5028000	216.00
RESERPINE	5	99.2060000	717.00
ESTRADIOL	9	100.0000000	130.00

APPENDIX H

DATA ON COMPOUNDS USED FOR EACH TEST OF CORRELATION BETWEEN MUTAGENICITY AND TUMORIGENICITY

NOTE- This appendix includes all of the data used for the tests of correlation between the assays for tumorigenicity and the short term tests. As noted in the text, and demonstrated in Appendix G, it was not always possible to calculate a median relative potency for all test compounds relative to all reference compounds. This obviously limits the analysis to those compounds for which it was possible to make the appropriate calculations for both mutagenicity and tumorigenicity. MEDIAN1 is the mutagenic median relative potencies and MEDIAN2 is the tumorigenic median relative potencies.

DATA STANDARDIZED TO 3-METHYLCHOLANTHRENE

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	0.0216000	0.5208000
DDT	0.1534000	0.1232000
B(a)P	0.9402000	1.0000000
RESERPINE	8.4160000	1.6600000
CARBON TETRACHLORIDE	0.0013300	0.5746000
DES	1.5384000	0.1619000
PHENOBARBITOL	0.0227000	0.1208000
PROPIOLACTONE	0.0175000	0.0537000
PHENACETIN	0.0002000	0.0199000
THIOACETAMIDE	0.0584000	0.0549000
THIOUREA	0.0086000	0.0503000
DMNA	0.4482000	0.0698000
METHYLMETHANE SULFONATE	0.0684000	0.0059000
CHLOROFORM	0.0071000	0.0183000
BENZENE	0.0024000	0.1513000
METHYLIODIDE	0.1204000	0.2649000
VINYL CHLORIDE	0.0267000	0.0000270
ETHYLENE OXIDE	0.0094000	0.0730000
DIMETHYLCARBAMOYL CHLORIDE	0.0015000	0.0622000
SACCHARINE	0.0029000	0.1747000
2-NAPTHYLAMINE	0.0186000	0.1147000
DICHLOROBENZIDINE	0.0222000	0.9440000
4-AMINOBIPHENYL	0.0583000	0.3153000
BENZIDINE	0.0039000	0.6865000
TOLUIDINE	0.0250000	0.0067000
DBCP	0.0035000	0.5032000
N-NITROSOPIPERIDINE	0.1825000	0.3189000
EPICHLOROHYDRIN	0.0082000	0.0154000
EDB	0.0015000	0.0345000
ACRYLONITRILE	0.0065000	0.0050000
BHT(BUTYLATEDHYDROXYTOLUENE)	0.0085000	32.9900000
ETHYLENEIMINE	0.2294000	0.0265000

DATA STANDARDIZED TO 3-METHYLCHOLANTHRENE (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
HYDRAZINE	0.0023000	0.0019000
CHORAMBUCIL	0.6302000	0.6079000
AZOTHIOPRINE	0.0177000	0.1034000
BCME	0.0172000	0.4291000
N-METHYL-N-NITROSOUREA	0.0994000	0.0244000
TCDD	6154.0000000	300.3818000
ACRYLAMIDE	0.0019000	0.7327000
CADMIUM CHLORIDE	2.4835000	0.9329000
NICKEL SUBSULFIDE	0.1289000	0.8955000
CIS-PLATIN	0.6952000	10.5696000
PHORBOL ACETATE	7.5003000	23.3206000

DATA STANDARDIZED TO BENZO(a)PYRENE [B(a)P]

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	0.4142000	0.0198000
ESTRADIOL	100.0000000	0.0123000
DDT	1.4047000	0.9283000
CARBON TETRACHLORIDE	0.2971000	0.0277000
3-MC	1.0000000	1.0634000
DES	0.3545000	10.9000000
PROPIOLACTONE	0.0571000	0.0028000
DMNA	0.0642000	0.1468000
METHYLMETHANE SULFONATE	0.0199000	0.1773000
BENZENE	0.0852000	0.0013000
METHYLIODIDE	0.4649000	0.1280000
VINYL CHLORIDE	0.0000020	0.0053000
ETHYLENE OXIDE	0.0100000	0.0019000
DIMETHYLCARBAMOYL CHLORIDE	0.2284000	0.0086000
SACCHARINE	0.0012200	0.0045000
2-NAPTHYLAMINE	0.2837000	0.0710000
DICHLOROBENZIDINE	0.6276000	0.0153000
4-AMINOBIPHENYL	0.1461000	0.0278000
BENZIDINE	0.3408000	0.0212000
TOLUIDINE	0.0086000	0.0119000
DBCP	0.2979000	0.0008400
N-NITROSOPIPERIDINE	0.0425000	0.0194000
EPICHLOROHYDRIN	0.4613000	0.0031000

DATA STANDARDIZED TO BENZO(a)PYRENE [B(a)P] (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
EDB	0.1984000	0.0089000
ETHYLENEIMINE	0.0606000	0.5082000
HYDRAZINE	0.0198000	0.0456000
CHORAMBUCIL	1.2063000	0.6702000
AZOTHIOPRINE	0.2015000	0.0137000
BCME	0.2723000	0.0070000
N-METHYL-N-NITROSOUREA	0.1220000	0.3168000
TCDD	20.9324000	391.9625000
ACRYLAMIDE	9.8412000	0.0066000
CADMIUM CHLORIDE	1.2460000	0.7879000
NICKEL SUBSULFIDE	0.3174000	0.0849000
N-NITROSONORNICOTENE	0.2732000	0.0080000
PHORBOL ACETATE	27.5028000	3.7155000

DATA STANDARDIZED TO BENZENE

TEST CHEMICAL	MEDIAN1	MEDIAN2
FORMALDEHYDE	0.5542000	80.0000000
CYCLOPHOSPHAMIDE	2.6769000	47.9391000
DDT	0.6269000	8.5574000
B(a)P	12.1077000	5993.0000000
RESERPINE	13.5281000	1121.0000000
CARBON TETRACHLORIDE	0.7643000	0.3949000
3-MC	44.6600000	412.3081000
DES	53.0000000	1606.0000000
PROPIOLACTONE	4.4000000	8.0270000
PHENACETIN	1.5777000	0.2086000
THIOACETAMIDE	0.1772000	3.6380000
THIOUREA	0.9743000	0.6496000
DMNA	0.6077000	221.1600000
METHYLMETHANE SULFONATE	2.6311000	85.7906000
CHLOROFORM	0.1000000	0.5341000
VINYL CHLORIDE	0.2748000	6.0000000
ETHYLENE OXIDE	0.0263000	5.7381000
DIMETHYLCARBAMOYL CHLORIDE	0.3619000	64.2298000
2-NAPTHYLAMINE	5.0511000	3.5209000
4-AMINOBIPHENYL	2.7516000	27.6391000

DATA STANDARDIZED TO BENZENE (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
BENZIDINE	4.7188000	903.0679000
N-NITROSOPIPERIDINE	0.1408000	24.2056000
EPICHLOROHYDRIN	11.0740000	1.0135000
EDB	3.5256000	3.6354000
ACRYLONITRILE	7.4013000	5.8240000
HYDRAZINE	0.5625000	152.0000000
ALDRIN	18.1627000	316.2987000
AZOTHIOPRINE	1.2190000	7.2847000
N-METHYL-N-NITROSOUREA	5.2821000	80.3312000
TCDD	470.0000000	1566784.0000000
ACRYLAMIDE	10.6838000	0.3720000

DATA STANDARIZED TO CADMIUM

TEST CHEMICAL	MEDIAN1	MEDIAN2
FORMALDEHYDE	0.0171000	0.0007300
B(a)P	0.8373000	1.7117000
3-MC	1.0720000	0.4027000
DES	0.0488000	10.4021000
PROPIOLACTONE	0.0233000	0.0505000
DMNA	0.1241000	0.0585000
ETHYLENE OXIDE	0.0022000	0.0001920
DIMETHYLCARBAMOYL CHLORIDE	0.0065000	0.0041000
DICHLOROBENZIDINE	2530.3644000	0.0015000
4-AMINOBIPHENYL	0.0676000	0.0013000
BENZIDINE	0.4167000	0.0069000
DBCP	0.0250000	0.0044000
N-NITROSOPIPERIDINE	2.2799000	0.0051000
EPICHLOROHYDRIN	0.0768000	0.0000820
EDB	0.2264000	0.0002700
ACRYLONITRILE	0.0005300	0.0002700
ETHYLENEIMINE	0.5000000	0.1507000
HYDRAZINE	0.0036000	0.0022000
N-METHYL-N-NITROSOUREA	0.0932000	0.1913000
NICKEL SUBSULFIDE	0.1440000	0.0448000

DATA STANDARDIZED TO CYCLOPHOSPHAMIDE

TEST CHEMICAL	MEDIAN1	MEDIAN2
DDT	0.5650000	0.1064000
B(a)P	2.4138000	87.8084000
RESERPINE	7.7800000	12.6659000
3-MC	1.9200000	46.2069000
DES	5.0334000	875.6914000
PHENOBARBITOL	0.0613000	0.0740000
PROPIOLACTONE	1.2000000	0.4826000
PHENACETIN	0.0224000	0.0042000
THIOACETAMIDE	0.0479000	0.0464000
THIOUREA	73.8130000	0.0092000
DMNA	0.2500000	4.7119000
METHYLMETHANE SULFONATE	0.8429000	6.2323000
CHLOROFORM	0.4559000	0.0083000
BENZENE	0.3736000	0.0209000
VINYL CHLORIDE	0.0625000	0.0082000
ETHYLENE OXIDE	0.1405000	0.1516000
DIMETHYLCARBAMOYL CHLORIDE	1.0000000	0.1862000
2-NAPTHYLAMINE	0.5479000	0.0444000
DICHLOROBENZIDINE	0.9846000	0.0521000
BENZIDINE	0.7050000	0.1134000
DBCP	0.7020000	0.0916000
N-NITROSOPIPERIDINE	0.3392000	0.4776000
EPICHLOROHYDRIN	0.2222000	0.0106000
EDB	1.2533000	0.0571000
ACRYLONITRILE	0.4190000	0.0494000
BHT(BUTYLATEDHYDROXYTOLUENE)	0.5057000	0.0033000
ALDRIN	0.4827000	6.5942000
AZOTHIOPRINE	1.3260000	0.2609000
TCDD	847.0000000	14983.0000000
ACRYLAMIDE	4.5467000	0.0409000

DATA STANDARDIZED TO DDT

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	1.8430000	9.4959000
B(a)P	0.7119000	14.9071000
3-MC	4.5424000	6.7757000
DES	8.5200000	210.1444000
PROPIOLACTONE	0.4100000	1.1450000
THIOACETAMIDE	0.1412000	0.3847000
DMNA	0.0000062	14.8528000
METHYLMETHANE SULFONATE	2.5760000	0.0756000
BENZENE	0.8196000	0.1192000
VINYL CHLORIDE	0.0000001	0.0623000
2-NAPTHYLAMINE	0.4520000	0.2045000
4-AMINOBIPHENYL	0.5000000	138.1207000
BENZIDINE	3.1350000	5.8956000
DBCP	0.6666000	0.5819000
EPICHLOROHYDRIN	9.4703000	0.0815000
EDB	0.0000093	0.3977000
BHT(BUTYLATEDHYDROXYTOLUENE)	0.5057000	0.0478000
ETHYLENEIMINE	1.4970000	0.2431200
ALDRIN	10.7852000	8.6000000
N-METHYL-N-NITROSOUREA	0.0219000	1.4354000
TCDD	24104.9991000	150725.0000000
ACRYLAMIDE	0.4473000	0.0568000

DATA STANDARDIZED TO DIETHYLSTILBESTROL (DES)

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	0.1987000	0.0314000
ESTRADIOL	1.0000000	0.6680000
DDT	0.1174000	0.0048000
B(a)P	2.8209000	0.0918000
CARBON TETRACHLORIDE	0.0557000	0.0000820
3-MC	7.2330000	0.6500000
PROPIOLACTONE	1.9571000	0.0081000
PHENACETIN	0.0125000	0.0000180
THIOACETAMIDE	0.0095000	0.0003300
DMNA	0.0083000	0.3263000
METHYLMETHANE SULFONATE	0.0748000	0.0003700

DATA STANDARDIZED TO DIETHYLSTILBESTROL (DES) (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
CHLOROFORM	0.0149000	0.0002800
BENZENE	0.0215000	0.0009800
ETHYLENE OXIDE	0.0035000	0.0346000
DIMETHYLCARBAMOYL CHLORIDE	0.1024000	0.0001600
2-NAPTHYLAMINE	0.4002000	0.0215000
4-AMINOBIPHENYL	6.1480000	0.0012000
BENZIDINE	0.2403000	0.0008000
N-NITROSOPIPERIDINE	0.1418000	0.0462000
EPICHLOROHYDRIN	0.0554000	0.0147000
EDB	0.7794000	0.0004000
ETHYLENEIMINE	0.0912000	0.0033000
ALDRIN	0.5118000	0.0328000
AZOTHIOPRINE	0.6201000	0.0466000
N-METHYL-N-NITROSOUREA	0.0547000	0.0077000
TCDD	10.4994000	8040.7300000
ACRYLAMIDE	1.2723000	0.0003000
CADMIUM CHLORIDE	20.4853000	0.0931000
N-NITROSONORNICOTENE	230.7690000	0.0008000

DATA STANDARDIZED TO DIMETHYLNITROSAMINE (DMNA)

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	4.0000000	0.2122000
ESTRADIOL	540.0000000	0.9189000
DDT	490676.0000000	0.0673000
B(a)P	14.0000000	6.8108000
RESERPINE	119.3320000	3.5052000
CARBON TETRACHLORIDE	0.4162000	0.0030000
3-MC	3.6217000	2.2634000
DES	120.6000000	3.0644000
PROPIOLACTONE	0.9729000	0.0363000
CHLORDANE	39.1891000	0.1178000
PHENACETIN	0.8030000	0.0011700
THIOACETAMIDE	0.1826000	0.0198400
HIOUREA	1.0271000	0.0029200
METHYLMETHANE SULFONATE	0.7432000	0.2382000
CHLOROFORM	0.2632000	0.0029000

DATA STANDARDIZED TO DIMETHYLNITROSAMINE (DMNA) (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
BENZENE	1.6888800	0.0045000
VINYL CHLORIDE	0.0023000	0.0064000
ETHYLENE OXIDE	0.0459000	0.0141000
DIMETHYLSULFATE	3.3750000	0.8332000
DIMETHYLCARBAMOYL CHLORIDE	0.2700000	0.0196000
SACCHARINE	0.1236000	0.0003000
2-NAPTHYLAMINE	3.1549000	0.0157000
DICHLOROBENZIDINE	42.5570000	0.0190000
4-AMINOBIPHENYL	2.2836000	0.1202000
BENZIDINE	3.0549000	0.0497000
TOLUIDINE	0.5642000	0.0888000
DBCP	0.9112000	0.0174700
N-NITROSOPIPERIDINE	0.4689000	0.1269000
EPICHLOROHYDRIN	7.1038000	0.0038000
EDB	1.9041000	0.0171000
ACRYLONITRILE	0.4337000	0.0107000
ETHYLENEIMINE	2.3243000	0.1978000
HYDRAZINE	0.3412000	0.0126000
CHORAMBUCIL	5.2145000	0.9968000
ALDRIN	45.0000000	1.1020000
AZOTHIOPRINE	0.3743000	0.0581200
BCME	7.7711000	0.2214000
N-METHYL-N-NITROSOUREA	1.0000000	0.9279000
TCDD	2116.3311000	6780.1566000
ACRYLAMIDE	15.1515000	0.0038000
CADMIUM CHLORIDE	8.3655000	17.0891000
NICKEL SUBSULFIDE	4.5000000	0.6687000
CIS-PLATIN	40.5409000	1.7513000
PHORBOL ACETATE	617.0935000	66.7025000

DATA STAMDARDIZED TO EPICHLOROHYDRIN

TEST CHEMICAL	MEDIAN1	MEDIAN2
FORMALDEHYDE	0.1075000	7.0679000
CYCLOPHOSPHAMIDE	4.5000000	93.9777000
DDT	0.1602000	12.3554000
B(a)P	2.1677000	420.0000000
3-MC	87.1776000	124.8144000
DES	18.0588000	1314.0000000

DATA STAMDARDIZED TO EPICHLOROHYDRIN (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
PROPIOLACTONE	8.0763000	8.0785000
PHENACETIN	0.4900000	0.7946000
THIOACETAMIDE	0.0230000	4.8387000
THIOUREA	0.1500000	0.8688000
DMNA	0.6341000	262.0421000
CHLOROFORM	1.2796000	0.9278000
BENZENE	0.0968000	0.9871000
VINYL CHLORIDE	0.0326000	1.0000000
ETHYLENE OXIDE	0.3751000	2.0000000
DIMETHYLCARBAMOYL CHLORIDE	3.5187000	0.7444700
2-NAPTHYLAMINE	1.5376000	4.1720000
DICHLOROBENZIDINE	81.4596000	5.5088000
BENZIDINE	2.9677000	37.7126000
DBCP	2.5268000	23.2571000
N-NITROSOPIPERIDINE	0.7629000	37.2339000
EDB	1.5752000	5.3728000
ACRYLONITRILE	16.3901000	5.3180000
HYDRAZINE	0.1000000	20.0000000
ALDRIN	0.6721000	887.7616000
AZOTHIOPRINE	0.2979000	10.6300000
BCME	9.2749000	166.1934000
N-METHYL-N-NITROSOUREA	1.3844000	53.1613000
TCDD	353.0000000	2017018.3700000
ACRYLAMIDE	20.8889000	4.1880000
CADMIUM CHLORIDE	13.5989000	13693.0000000

DATA STANDARDIZED TO METHYLMETHANE SULFONATE

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	1.1864000	0.2200000
ESTRADIOL	304.4472000	12.7900000
DDT	0.4054000	13.2202000
B(a)P	45.9900000	5.6392000
CARBON TETRACHLORIDE	1.3333000	0.3403000
3-MC	17.8700000	14.6182000
DES	13.3667000	2702.5100000
PROPIOLACTONE	0.9359000	2.7900000
PHENACETIN	0.2644000	0.0012000
THIOACETAMIDE	0.1716000	0.4019000

DATA STANDARDIZED TO METHYLMETHANE SULFONATE (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
DMNA	1.3454000	4.3200000
CHLOROFORM	0.5409000	0.3223000
BENZENE	0.3800000	0.0117000
VINYL CHLORIDE	0.0573000	1.9325000
DIMETHYLSULFATE	1.0000000	2.9700000
2-NAPTHYLAMINE	1.3000000	0.0720000
DICHLOROBENZIDINE	192.7700000	0.0600000
4-AMINOBIPHENYL	7.6818000	0.2350000
N-NITROSOPIPERIDINE	0.3200000	0.1960000
EDB	1.1280000	0.9470000
BHT(BUTYLATEDHYDROXYTOLUENE)	12.9000000	1.3793000
ETHYLENEIMINE	0.5746000	3.5963000
ALDRIN	17.6000000	6.9681000
N-METHYL-N-NITROSOUREA	1.1492000	2.8091000
TCDD	731.8400000	581019.0000000
ACRYLAMIDE	20.6700000	0.1746000
NICKEL SUBSULFIDE	23.9998000	5.4500000

DATA STANDARDIZED TO 2-NAPTHYLAMINE

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	1.8252000	22.5331000
DDT	2.2400000	4.8899000
B(a)P	3.5245000	14.6853000
RESERPINE	1.5000000	285.8877000
CARBON TETRACHLORIDE	3.4460000	0.4142000
3-MC	10.9500000	53.5464000
DES	2.8100000	1417.3240000
PHENOBARBITOL	1.7762000	1.9821000
PROPIOLACTONE	2.5175000	2.3937000
PHENACETIN	0.7586000	0.0802000
THIOACETAMIDE	0.0540000	1.1509000
THIOUREA	0.7600000	0.2126000
DMNA	0.3340000	63.6902000
METHYLMETHANE SULFONATE	1.1500000	13.8888000
CHLOROFORM	0.1041000	0.2080000
BENZENE	0.1980000	0.4306000
VINYL CHLORIDE	0.0007300	2.1409000
ETHYLENE OXIDE	0.2501000	0.7825000

DATA STANDARDIZED TO 2-NAPTHYLAMINE (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
DIMETHYLCARBAMOYL CHLORIDE	1.3200000	0.2324000
DICHLOROBENZIDINE	2.5600000	1.3480000
4-AMINOBIPHENYL	1.0000000	5.2490000
BENZIDINE	1.2867000	3.7230000
DBCP	5.6863000	2.5787000
N-NITROSOPIPERIDINE	0.3493000	7.4026000
EPICHLOROHYDRIN	0.6503000	0.2340000
EDB	3.6232000	1.4550000
ACRYLONITRILE	0.2965000	1.3237000
ETHYLENEIMINE	0.3804000	27.7569000
ALDRIN	1.3000000	176.4991000
AZOTHIOPRINE	0.0255000	2.8175000
BCME	4.0210000	5.1468000
N-METHYL-N-NITROSOUREA	0.7202000	15.3659000
TCDD	44.1621000	616993.7000000
ACRYLAMIDE	6.1969000	1.0058000

DATA STANDARDIZED TO N-METHYL-N-NITROSOUREA

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	2.5340000	0.0650000
ESTRADIOL	750.0000000	0.1886000
DDT	45.5809000	0.6967000
B(a)P	8.0000000	3.1600000
CARBON TETRACHLORIDE	0.7408000	0.0032000
3-MC	40.9090000	10.0622000
DES	18.2726000	130.0986000
PROPIOLACTONE	0.3495000	0.0539000
DMNA	0.7184000	1.0777000
METHYLMETHANE SULFONATE	0.8808000	0.3560000
BENZENE	0.1893000	0.0124000
VINYL CHLORIDE	0.1958000	0.3045000
DIMETHYLSULFATE	0.7692000	0.6116000
DIMETHYLCARBAMOYL CHLORIDE	1.7690000	0.0205000
2-NAPTHYLAMINE	1.3883000	0.0651000
DICHLOROBENZIDINE	49.0353000	0.0181000
4-AMINOBIPHENYL	1.6407000	0.1632000
BENZIDINE	1.1148000	0.0576000
DBCP	0.6546000	0.0070000

DATA STANDARDIZED TO N-METHYL-N-NITROSOUREA (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
N-NITROSOPIPERIDINE	1.6640000	0.0814000
EPICHLOROHYDRIN	0.7226000	0.0188000
EDB	2.9204000	0.0273000
ETHYLENEIMINE	0.4089000	0.5329000
CHORAMBUCIL	22.3000000	5.3290000
AZOTHIOPRINE	0.0482000	0.1681000
BCME	3.8703000	0.1400000
TCDD	1586.0000000	15433.0000000
ACRYLAMIDE	32.3200000	0.0027000
CADMIUM CHLORIDE	10.8543000	14.7288000
NICKEL SUBSULFIDE	10.8544000	2.3300000
CIS-PLATIN	51.5370000	2.2468000
N-NITROSONORNICOTENE	25.5857000	0.0391000
PHORBOL ACETATE	153.8461000	149.3731000

DATA STANDARDIZED TO PROPIOLACTONE

TEST CHEMICAL	MEDIAN1	MEDIAN2
FORMALDEHYDE	0.4167000	0.0595000
DDT	2.4450000	0.8756000
B(a)P	17.5000000	361.6670000
CARBON TETRACHLORIDE	7.2300000	0.0127000
3-MC	18.6108000	57.0000000
DES	0.7240000	136.9000000
PHENACETIN	0.0932000	0.0125000
THIOACETAMIDE	0.9680000	0.4279000
THIOUREA	0.3500000	0.0804000
DMNA	1.0277000	27.4975000
METHYLMETHANE SULFONATE	1.0685000	5.3076000
CHLOROFORM	0.0331000	0.0617000
BENZENE	0.2272000	0.1246000
VINYL CHLORIDE	0.0003000	0.7132000
ETHYLENE OXIDE	0.0408000	0.3202000
DIMETHYLCARBAMOYL CHLORIDE	0.3750000	0.4672000
2-NAPTHYLAMINE	0.3972000	0.4223000
DICHLOROBENZIDINE	1.8630000	0.5359000
4-AMINOBIPHENYL	0.3559000	0.7498000
BENZIDINE	1.2770000	0.1503000
DBCP	1.2500000	0.6417000

DATA STANDARDIZED TO PROPIOLACTONE (continued)

TEST CHEMICAL	MEDIAN1	MEDIAN2
N-NITROSOPIPERIDINE	2.0450000	2.0117000
EPICHLOROHYDRIN	0.1391000	0.1238000
EDB	1.5000000	0.4700000
ETHYLENEIMINE	0.3056000	7.8274000
AZOTHIOPRINE	0.0000140	1.0128000
BCME	1.5971000	1.7056000
N-METHYL-N-NITROSOUREA	3.0300000	19.9300000
TCDD	34.7220000	447330.0000000
ACRYLAMIDE	10.3333000	0.0510000
CADMIUM CHLORIDE	58.0000000	20.2214000
PHORBOL ACETATE	6173.0000000	13092.0000000
BHA(BUTYLATEDHYDROXYANISOLE)	392.8346000	0.0283000

DATA STANDARDIZED TO TCDD

TEST CHEMICAL	MEDIAN1	MEDIAN2
CYCLOPHOSPHAMIDE	0.0014860	0.0000699000
DDT	0.0001900	0.0000066350
B(a)P	0.0660000	0.0021740000
3-MC	0.0033000	0.0001625000
DES	0.4414000	0.0011840000
PROPIOLACTONE	0.0288000	0.0000022360
THIOACETAMIDE	0.0009000	0.0000022900
DMNA	0.0016800	0.0001475000
METHYLMETHANE SULFONATE	0.0014000	0.0000216000
BENZENE	0.0050000	0.0000004891
VINYL CHLORIDE	0.0000400	0.0000005947
ETHYLENE OXIDE	0.0785000	0.0000028070
2-NAPTHYLAMINE	0.0298000	0.0000016880
4-AMINOBIPHENYL	0.2677000	0.0000569500
N-NITROSOPIPERIDINE	0.0035000	0.0000187200
EPICHLOROHYDRIN	0.0368000	0.0000004805
EDB	0.1887000	0.0000023160
ACRYLONITRILE	0.4115000	0.0000022610
ALDRIN	0.1519000	0.0001906000
N-METHYL-N-NITROSOUREA	0.0006300	0.0000648000
ACRYLAMIDE	9.6268000	0.0000002161
PHORBOL ACETATE	11.9162000	0.0080670000

Table 7. Correlation of tumorigenicity data using different references[a]

	Propiolactone (Direct)	DMNA (Indirect)	Benzene (Epigenetic)	TCDD (Epigenetic)	Epichlorohydrin (Epigenetic)	Cadmium (Metal)
Propiolactone (Direct)						
DMNA (Indirect)	N[b] = 36 p < .001[c]					
Benzene (Epigenetic)	N = 34 p < .001	N = 36 p < .001				
TCDD (Epigenetic)	N = 31 p < .001	N = 35 p < .001	N = 32 p < .001			
Epichlorohydrin (Epigenetic)	N = 32 p < .001	N = 34 p < .001	N = 34 p < .001	N = 32 p < .001		
Cadmium (Metal)	N = 20 p < .001	N = 22 p < .001	N = 22 p < .001	N = 17 p < .05	N = 19 p < .001	

[a]References have been selected to represent members of the major classes of theorized mechanisms of action.

[b]Number of compounds which had sufficient information for analysis.

[c]As with the other tests of correlation, the Spearman Rank Correlation Coefficient was transformed to a t statistic and tested against a two tailed distribution.

Table 8. Correlation of mutagenicity data using different references[a]

	Propiolactone (Direct)	DMNA (Indirect)	Benzene (Epigenetic)	TCDD (Epigenetic)	Epichlorohydrin (Epigenetic)	Cadmium (Metal)
Propiolactone (Direct)						
DMNA (Indirect)	N[b] = 41 p < .001[c]					
Benzene (Epigenetic)	N = 37 p < .001	N = 41 p < .001				
TCDD (Epigenetic)	N = 30 p < .5	N = 32 p < .05	N = 32 p < .005			
Epichlorohydrin (Epigenetic)	N = 37 p < .001	N = 39 p < .001	N = 35 p < .001	N = 31 p < .001		
Cadmium (Metal)	N = 36 p < .025	N = 39 p < .01	N = 36 p < .025	N = 30 p < .5	N = 36 p < .05	

[a]References have been selected to represent members of the major classes of theorized mechanisms of action.

[b]Number of compounds which had sufficient information for analysis.

[c]As with the other tests of correlation, the Spearman Rank Correlation Coefficient was transformed to a t statistic and tested against 1 a two tailed distribution.

END

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