Animal Carcinogenicity Studies: Obstacles to Human Extrapolation

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Abstract

Due to a paucity of human exposure data, risk classification and the consequent regulation of exposures to potential carcinogens has traditionally relied heavily upon animal tests. However, several investigations have revealed animal carcinogenicity data to be lacking in human specificity (the ability to distinguish human from animal carcinogens, where different). In order to investigate the reasons, we surveyed the chemicals possessing animal but not human exposure data within the U.S. Environmental Protection Agency chemicals database that had received human carcinogenicity assessments. We found a wide variety of species used, with rodents being predominant; a wide variety of routes of administration used, and a particularly wide variety of organ systems affected. The likely causes of the poor human specificity, and hence utility, of rodent carcinogenicity bioassays include (i) the profound discordance of bioassay results between rodent species, strains and genders, and further, between rodents and human beings; (ii) the variable and substantial stresses caused by handling and restraint and the stressful routes of administration endemic to carcinogenicity bioassays, with consequent effects on hormonal regulation, immune status and carcinogenesis predisposition; (iii) the differences in transport mechanisms and rates of absorption between test routes of administration and other important human routes of exposure; (iv) the considerable variability of organ systems in response to carcinogenic insults, between and within species, combined with the inability of commonly-used predictors of human carcinogenicity, such as the number of organ systems or sex-species groups effected, or fatalities, to withstand careful scrutiny; and (v) the inherent predisposition of chronic high dose bioassays towards false positive results, due to the overwhelming of physiological defences, and the unnatural elevation of cell division rates during *ad libitum* feeding studies. Such factors render attempts to extrapolate accurate human carcinogenicity assessments from animal data profoundly difficult, if not impossible.

Introduction

Due to a paucity of human exposure data, the regulation of human exposures to chemicals by regulatory authorities such as the U.S. Environmental Protection Agency (EPA) relies heavily upon animal carcinogenicity tests. The environmental contaminants of greatest U.S. concern are listed in the EPA's Integrated Risk Information System (IRIS) chemicals database. However, our survey of the 160 IRIS chemicals lacking significant human exposure data but possessing animal data as of January 1, 2004, found that the EPA considered the animal data inadequate to support the substantially useful classifications of probable human carcinogen or non-carcinogen in the majority (58.1%; 93/160) of cases.

The sensitivity of the traditional rodent bioassay to human carcinogens (ability to detect them) for *some* sex-species combination is not in question. However, our study and those of others clearly demonstrate its poor human specificity (ability to distinguish human from animal carcinogens, where different), which greatly undermines its human predictivity. To investigate the reasons for this inadequacy, we examined the animal test data for these 160 IRIS chemicals.

Methods

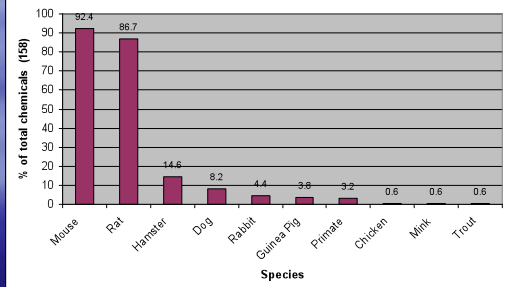
Of the 543 chemicals contained within the EPA's IRIS chemicals database, as of Jan. 1, 2004, 160 lacked significant human exposure data but possessed animal data, and had received human carcinogenicity assessments. For each of these we determined the species and route(s) of administration used, and the organ system(s) affected.

Results

Species used

At least 10 different species were used, namely: chickens, dogs, guinea pigs, hamsters, mice, mink, primates (one macaque, three unspecified "monkey" species, and one unspecified "primate" species), rabbits, rats, and trout. The three species most commonly used were mice (92.4%), rats (86.7%), and hamsters (14.6%) (Figure 1).

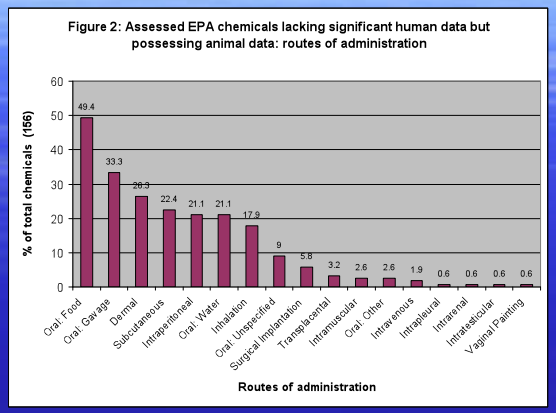
Figure 1: Assessed EPA chemicals lacking significant human data but possessing animal data: species used



Routes of administration

Twelve non-oral routes of administration, and a variety of oral routes, not always specified, were used. They were: dermal, inhalation, intramuscular, intraperitoneal, intrapleural, intrarenal, intratesticular, intravenous, oral: food, oral: gavage, oral: water, oral: other (eg, capsule, toothpaste additive), oral: unspecified, subcutaneous, surgical implantation, transplacental, and vaginal painting. Those most commonly used were food (49.4%), gavage (33.3%), and dermal administration (26.3%). Other routes of major interest were drinking water (21.1%), and inhalation (17.9%) (Figure 2).

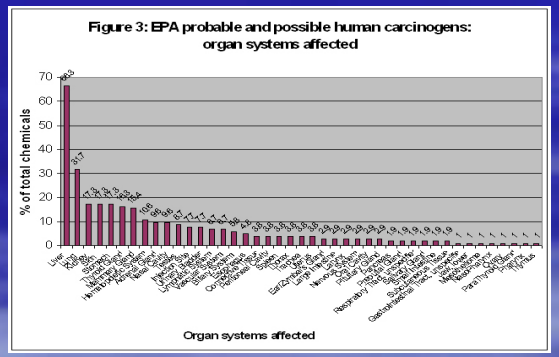
Figure 2: Assessed EPA chemicals lacking significant human data but possessing animal data: routes of administration



Organs affected

Up to 43 organ systems were found to exhibit neoplastic lesions, with up to 11 jointly affected for each chemical. The systems most commonly affected were the liver (66.3%), the lung (31.7%), and the kidney, skin and stomach (all 17.3%) (Figure 3).

Figure 3: EPA probable and possible human carcinogens: organ systems affected



Discussion

A wide variety of species were used, with rodents being predominant; a wide variety of routes of administration were used, and a particularly wide variety of organ systems were affected. Key biological and mathematical obstacles to accurate extrapolation of human carcinogenicity from such animal data include:

Discordance between mice and rats

Large-scale studies have revealed that chemicals carcinogenic in mice are not so in rats, and in males are not so in females, and vice-versa. In fact, only around a quarter of rodent carcinogens are consistently carcinogenic across all sex-species groups. Even within the same sex-species group, many chemicals yield inconsistent results.

Discordance between rodents and primates

Numerous important differences between rodents and humans impact on carcinogenesis predisposition, such as lifespan (2.5 vs. 70 yrs), food consumption (50 vs. 10 g/kg/day), basal metabolic rate (109 vs. 26 kcal/kg/day), anatomic differences (the forestomach, Zymbal's gland, Harderian gland, preputial gland and clitoral gland exist only in the rat), stomach pH (4-5 vs. 1-2), and, very significantly, DNA excision repair rates (low vs. high). Additionally, quantitative or qualitative differences in absorption, distribution, metabolism and elimination pathways or rates can all influence the carcinogenicity of a chemical.

The high carcinogenesis predisposition of rodents when compared to primates complicates extrapolation of results to humans. It is remarkable that mice can develop very malignant tumors with multiple genetic alterations within 6-18 months, whereas aggressive tumors in humans or other primates may take many years to reach an equivalently life-threatening stage.

Two 26 and 32 year studies of rodent carcinogens revealed that less than half were monkey carcinogens. However, some 50% of all chemicals tested for carcinogenicity in rodents are positive in at least one experiment, with carcinogenesis predisposition even higher in some commonly used strains. Holliday (1996) suggested that the high rodent carcinogenesis predisposition when compared to humans might be due to less efficient DNA repair, poorer control of genetic stability, and/or altered control of gene expression. The high doses used in bioassays may also increase apparent carcinogenicity (see following).

Carcinogenesis predisposition of stressful routes of administration

Studies of mice, rats, hamsters, monkeys, dogs, rabbits, birds, and bats have shown that routine procedures such as handling and gavaging (insertion of a stomach tube for the oral administration of a test compound), cause significant increases in stress indicators, including concentrations of corticosterone (a stress hormone), glucose, growth hormone, norepinephrine, prolactin, thyroid-stimulating hormone, and triiodothyronine. Other blood measures, including packed cell volume, hemoglobin, and plasma protein, also rise significantly. These stress-related responses generally occur with every exposure to such a stressor; laboratory animals do not readily habituate to them.

Stress-related responses are particularly important in long-term carcinogenicity studies, in light of their heavy emphasis on stressful routes of administration. Of our applicable EPA chemicals, gavaging was used for 33.3%, and dermal administration (requiring handling and restraint) was used for 26.3% (Figure 2). Other routes of administration requiring handling and restraint as a minimum were intramuscular, intraperitoneal, intrapleural, intrarenal, intratesticular, intravenous, oral other than food or water (e.g., via capsule or toothpaste additive), subcutaneous, surgical implantation, and vaginal painting.

The stress-mediated hormonal changes that occur in response to such stressful stimuli predispose to immunosuppression and increased susceptibility to virtually all pathologies, including neoplasia.

Route-to-route extrapolation

Judgments frequently need to be made about the carcinogenicity of a chemical via a route of exposure different to that studied. For example, exposures of interest may be through inhalation of a chemical tested primarily through feeding studies. Given that only 17.9% of these chemicals were tested via inhalation, in contrast to the percentages tested via food (49.4%), gavaging (33.3%) or drinking water (21.1%), such dilemmas are hardly unlikely. However, the differences in transport mechanisms and rates of absorption between routes (e.g. oral, inhalation, dermal) can be great.

Organs affected

The wide variation in organ systems affected may have been exacerbated by the considerable carcinogenic variability of many chemicals between organ systems and species. Comparisons between mice, rats, hamsters and humans, for example, reveal that carcinogens are carcinogenic at the same site in another of these species no more than 50% of the time, severely complicating attempts to interpret the significance for humans of tumors in various locations.

Dose-related toxicity

Carcinogenicity bioassays typically rely upon the maximum tolerated dose (MTD), as indicated by increasing toxicity-related effects, in order to maximize their sensitivity to carcinogenic effects. However, prolonged exposure to high chemical doses can result in chronic irritation, cellular killing, and consequent cellular proliferation. Additionally, animals have a broad range of general physiological defences, such as epithelial shedding and inducible enzymes, which commonly prove effective at environmentally relevant doses, but which may be overwhelmed at higher doses. Combined with insufficient rest intervals between doses for DNA and tissue repair mechanisms to effectively operate, these factors can predispose to carcinogenesis.

Caloric-related mitogenesis

Ad libitum ('at will') feeding, as occurs in many studies, can also unnaturally elevate cell division. However, reviews of both the experimental and epidemiological literature show a high correlation between increased cell division and carcinogenesis.

Conclusions

The likely causes of the poor human specificity, and hence predictivity, of rodent carcinogenicity bioassays demonstrated by other investigators and ourselves, include (i) the profound discordance of bioassay results between rodent species, strains and genders, and further, between rodents and human beings; (ii) the variable and substantial stresses caused by handling and restraint and the stressful routes of administration endemic to carcinogenicity bioassays, with consequent effects on hormonal regulation, immune status and carcinogenesis predisposition; (iii) the differences in transport mechanisms and rates of absorption between test routes of administration and other important human routes of exposure; (iv) the considerable variability of organ systems in response to carcinogenic insults, between and within species, combined with the inability of commonly-used predictors of human carcinogenicity, such as the number of organ systems or sex-species groups effected, or fatalities, to withstand careful scrutiny; and (v) the inherent predisposition of chronic high dose bioassays towards false positive results, due to the overwhelming of physiological defences, and the unnatural elevation of cell division rates during *ad libitum* feeding studies. Such render attempts to extrapolate accurate human carcinogenicity assessments from animal data profoundly difficult, if not impossible.

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References

Available on request.