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Comment

The Importance of Data on Mechanism of Carcinogenesis in Efforts to Predict Low-Dose Human Risk[1]

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1. IMPLICATIONS OF THE FINDING THAT POTENCY ESTIMATES ARE CONSTRAINED TO A NARROW RANGE SURROUNDING THE MTD

The literature reviewed by Krewski *et al.* identifies validity problems associated with using the limited data from rodent bioassays in efforts to assess carcinogenic risk to humans. Krewski *et al.* discuss the debate as to whether the good correlation of carcinogenic potencies found between rats and mice should be interpreted as a justification for quantitative extrapolation from rodents to humans. In 1985, Bernstein *et al.*[(1)] showed that the observed correlation is largely artifactual, as follows: For chemicals that test positive in rodent bioassays, potency estimates based on the one-hit model are constrained to a narrow range surrounding the high dose tested, the maximum tolerated dose (MTD) (unless all dosed animals develop tumors, which rarely occurs). Over large numbers of chemicals, the MTDs for rats and mice are highly correlated and span many orders of magnitude. Hence, the potency correlation between rats and mice follows statistically. A debate followed in several papers. In their review, Krewski *et al.* report that potency and MTD are highly correlated, regardless of whether the potency estimate uses the one-stage, multistage, or Weibull model. Freedman, Gold, and Stone [(2)] recently examined how much of the observed correlation in potencies between species is artifactual. Our analysis involved two statistical models where the impacts of various assumptions could be calculated. In effect, the first model assumes that interspecies correlation of potencies is purely artifactual; it ignores the correlation between rats and mice of (potency × MTD), which is a rough measure of tumor yield. The second model incorporates the correlation in (potency × MTD) between rats and mice, which indicates that part of the interspecies correlation in potencies is real. A comparison of the models and data suggests that more than 80% of the interspecies correlation in carcinogenic potencies for chemicals positive in both rats and mice can be explained by the interspecies correlation in toxicity (MTD) and the correlation between log potency and log MTD. Thus, we have confirmed the findings of Bernstein *et al.* and conclude that while there may be some basis for extrapolation from rodents to humans, the interspecies correlation of potencies does not say much about the validity of that extrapolation.

Standard practice in regulatory risk assessment for a given rodent carcinogen is to extrapolate from the high doses of rodent bioassays to the low doses of most human exposures by multiplying carcinogenic potency in rodents by human exposure. However, since potency estimates are constrained to lie within a narrow range about the MTD, the usual "one-in-a-million risk" can be approximated merely by knowing the MTD. The striking implication of this fact is that the dose usually estimated by regulatory agencies to give one cancer in a million based on the linearized multistage model, can be approximated simply by dividing the MTD of a given rodent carcinogen by 380,000. The estimates for 90% of the chemicals are within a factor of 10 of that number.[(3)] From a toxicological perspective, extrapolating 380,000 times below the bioassay dose and treating all chemicals the same, is inadequate and can frequently be misleading. I concur with the conclusions of Krewski *et al.* that "correlations between the MTD and measures of cancer potency reflect the limited amount of information on cancer risks provided by carcinogen bioassay data," and that "If progress in carcinogenic risk assessment based on bioassay data is to be made, it seems that additional information beyond that contained in traditional experiments is required."

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2. THE NECESSITY AND FEASIBILITY OF USING MECHANISTIC DATA IN RISK ASSESSMENT

An important fact is being neglected in the conduct and interpretation of bioassays: In mutagenesis (and thus carcinogenesis) cell division is critical for converting DNA lesions to mutations.[4,5] Compelling theoretical reasons, as well as data from a large body of experiments,[4–6] indicate that prediction of carcinogenic risk to humans at low dose must take cell division into account. Cell division can be caused by high doses of chemicals in rodent bioassays (e.g., by chronic cell killing and consequent cell replacement or by suppression of intercellular communication). To the extent that increases in tumor incidence in rodent studies are due to the secondary effects of administering high doses, any chemical that increases cell division may be a rodent carcinogen.

Just evaluating a chemical as a rodent carcinogen without considering dose and mechanism of action can be fundamentally misleading for low-dose risk assessment. All chemicals, whether mutagens or not, can cause cell division at high doses, and this is not predictable from the structure of chemical. Mutagens can cause DNA lesions, but at high doses they can also cause cell killing and cell replacement, giving a multiplicative factor for mutagenesis; if the cell does not divide, then DNA lesions can be repaired out. If cell division is a dominant factor in carcinogenesis at the MTD, then at low doses where cell division is not generally induced, the hazards to humans of rodent carcinogens may be much lower than commonly assumed. Defenses are inducible at low doses, and even for mutagens it may be that the increment in DNA damage over the enormous rate of endogenous background damage may not be significant. Many nonmutagens will have a threshold, and there will be no risk at low dose.

Several of our findings in large-scale analyses of the results of animal cancer tests,[7] are consistent with the idea that cell division increases the carcinogenic effect in high dose bioassays, including: the high proportion of chemicals that are positive; the high proportion of rodent carcinogens that are not mutagenic; the fact that mutagens, which can both damage DNA and increase cell division at high doses, are more likely than nonmutagens to be positive, to induce tumors in both rats and mice, and to induce tumors at multiple sites. Analyses of the limited data on dose-response in bioassays are consistent with the idea that cell division from cell-killing and cell replacement is important. In the usual experimental design of dosing at the MTD and half MTD, both doses are high and may result in cell division. Even at these two high doses, 44% of the positive sites in NTP bioassays are statistically significant at the MTD but not at half the MTD[7] (See also Ref. 8). Theoretical analysis[9] and experimental work on dose-response (e.g., formaldehyde, diethylnitrosamine, 2-acetylaminofluorene) are also consistent with an important role for cell division.

It is clear that the mechanisms of action for all rodent carcinogens are not the same. For some chemicals there is evidence to support cell division effects unique to high doses (e.g., melamine and saccharin), and thus there appears to be a threshold. For others (e.g., butadiene and 2-acetylaminofluorene), there may well be multiplicative effects due to an interaction of cell division and DNA damage, but carcinogenic effects have been found considerably below the MTD. Sometimes, the mechanism leading to cell division and carcinogenesis in a rodent species has no analogy in humans (e.g., kidney tumors in male Fischer rats due to $\alpha_{2\mu}$-globulin). Studies of mechanism in rodent bioassays would help to clarify such differences.

Since the results of animal cancer tests are routinely used in risk assessments and regulatory policy, the best science and technology available should be used in interpreting those results. As currently conducted, rodent bioassays do not provide the information necessary to extrapolate from high to low dose. Adding routine measurements of cell division to the 90-day prechronic study for each test agent would provide information that would improve dose-setting, the interpretation of experimental results, and risk assessment. It would also be of particular interest to reevaluate some of the rodent carcinogens that are receiving extensive regulatory attention on the basis of standard risk assessment methodology (e.g., chloroform, trichloroethylene, and dioxin). Measurement of cell division at and below bioassay doses in subchronic studies for these chemicals would permit a reinterpretation of the rodent data and an improved assessment of the potential risk to humans at low dose.

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