Dose-Response Analysis of in vivo Mutagenicity Data Can Inform Cancer Risk Assessment

TERA, NCTR & Environ: a collaborative partnership to improve cancer risk assessment

ABSTRACT

Quantitative cancer risk assessments typically utilize high-dose rodent tumor data. Under the new EPA Cancer Risk Assessment Guidelines, the quantitative model chosen for cancer risk assessment is based on the mode-of-action (MOA) of the chemical under consideration. In particular, it is based on whether the weight of evidence supports the chemical causing tumors through a DNA-reactive MOA or another MOA. It is assumed that DNA-reactive carcinogens (sometimes also called mutagenic carcinogens, although the latter is a broader term) have low-dose linear dose-response curves. This default assumption of linearity for such carcinogens is widely debated but, to date, there has been insufficient evidence to resolve the issue. Traditionally, the assessment of mutagenicity and of genotoxicity has been a simple "yes/no" determination. We are currently evaluating the potential for a more complete analysis of *in vivo* mutagenicity data, including dose-response analysis, to inform the risk assessment process and support the choice of a low-dose extrapolation model. Dose-response analysis of mutagenicity data can provide estimates of the frequency of gene mutations per target tissue at various dose levels, including those corresponding to the doses in the tumor assays. By examining these estimates and comparing them to the observed frequency of tumors, one can ascertain if an assumption of a mutagenic precursor appears to be consistent with the tumor response. Moreover, benchmark dose (BMD) estimates can be derived directly from the mutagenicity dose-response models, and compared to BMDs for tumor endpoints from other studies. The essence of our proposed approach involves generating carefully designed *in vivo* mutagenicity dose-response data in the tumor target tissue with doses selected based on the tumor dose-response. We intend to use the Big Blue® rat, mouse, or other transgenic *in vivo* shuttle vector model. The approach is based on the assumption that if the chemical causes cancer via a mutagenic MOA three basic predictions exist:

- **The frequency of induced mutations per target tissue will be higher than the frequency of tumors per target tissue.**
- **Mutations will be observed prior to the observation of tumors (temporal relationship).**
- **If a chemical is a mutagenic carcinogen, mutations should be induced at doses lower than those required to form tumors (dose-response).**

Preliminary collaborative work using published data (Allen et al., 2005, and additional analyses described below) supports the potential utility of this approach.

BACKGROUND

Cancer is a disease of mutation and cell proliferation. Chemicals can induce cancer either by inducing new mutations or by increasing cell proliferation, which in turn increases the frequency of spontaneous mutations. Chemicals that induce new mutations are "mutagenic" carcinogens (sometimes called genotoxic carcinogens, but genotoxicity includes mechanisms beyond direct DNA reactivity and mutagenesis). Chemicals that merely cause cell proliferation will cause an increased number of mutant cells, thus allowing for the additional events that eventually result in tumor formation. These are "non-DNA-reactive" carcinogens.

Currently, a chemical is defined as a "mutagenic" carcinogen if it induces mutations in one of a number of validated test systems. Scientists have historically considered the totality of genotoxicity data in such decisions, but EPA's 2005 guidelines focus on DNA reactivity and direct mutagenic activity. While it is generally assumed that chemicals that induce mutations in these various systems will induce mutations in target tissues, this is not necessarily true. Many of the standard mutation assays are conducted *in vitro* and they may not reflect the *in vivo* situation. In rare cases there is *in vivo* mutation data in the specific tissue in which tumors are formed. While evidence that a chemical induces mutations in the target tissue certainly increases the weight-of-the-evidence that it is a mutagenic carcinogen, it should be noted that the induction of mutations in a target tissue does not necessarily mean that the chemical induces cancer exclusively via a mutational mechanism. It is possible that the chemical induces both mutagenic and other types of effects (such as cell proliferation) or that the tumor is mediated entirely or primarily through a non-mutagenic mechanism.

Identification of the mutation(s) directly responsible for the etiology of the tumor provides the ultimate evidence of whether a chemical is a mutagenic carcinogen. Acquiring this evidence, however, requires detecting all the possible "needles in a haystack" that could be involved in the direct tumor pathway. While this will be possible at some future time, we do not currently have the technology to detect all of the possible rare mutational events.

At the present time, it is necessary to use reporter genes, that is, genes not involved in tumor initiation and development but which detect the same types of mutations known to be involved in tumor initiation and development. *In vivo* mutation assays that can be used for this analysis include those using endogenous genes in lymphocytes (*Hprt* and *Tk*) and those using transgenic shuttle vectors (*lacI*, *cII* and Φ*X174am3*). These later assays have the advantage that they can be conducted in any tissue from which the DNA can be extracted.

We are currently examining dose-response modeling approaches for these reporter gene mutation markers. The BMD approach has generally been used for "non-cancer" endpoints. However, it is equally applicable to cancer endpoints including cancer-relevant reporter gene mutations. In fact, derivation of an effective dose for use in cancer risk assessment is equivalent to derivation of a benchmark dose. In all of these contexts, the BMD is a dose corresponding to a response rate of interest. The response rate (or rates in some instances) is specified *a priori* based on the needs of the assessment, and dose-response modeling is used to derive estimates of the dose corresponding to that response rate. Often statistical lower bounds on the dose estimate (sometimes designated as the BMDL) are used for subsequent risk assessment derivations [e.g., in establishing the point of departure for a cancer assessment, or in setting a reference dose (RfD) or reference concentration (RfC)].

PREDICTIONS

If a chemical causes cancer via a mutagenic MOA there are three basic predictions.

- The frequency of induced mutations per target organ will be higher than the frequency of tumors per target organ
- Mutations will be observed prior to the observation of tumors
- If a chemical is a mutagenic carcinogen, mutations should be induced at doses lower than those required to form tumors

An evaluation of these three expectations should be helpful in determining whether a carcinogen is a mutagenic carcinogen.

Initiating Mutation	Multi-stage events ► ► ►	Tumor
Mutants Per Target Tissue	>	Tumors Per Target Tissue
Mutations	Before	Tumors
Lowest Dose for Mutations	<	Lowest Dose for Tumors

TWO CASE STUDIES:

We present two case studies based on the 3rd prediction. Both of these case studies were developed by comparing published gene mutation data with published tumor data in the same background strain. Mutation was measured using the Big Blue transgenic mouse or rat and the target tissue for tumor induction. If mutational events are only observed at doses higher than those required to form tumors, then the MOA may be non-mutagenic. An observation that mutational events are induced at doses lower than those required to form tumors is consistent with a mutagenic MOA. This quantitative modeling approach should add to the weight-of-the evidence MOA assessment.

Dose-Response Modeling for Benchmark-Dose Estimation

Dose-response models were fitted to the cancer incidence data and mutant frequency data on each chemical for the purpose of estimating the 10% benchmark dose (BMD_{10}) and its 95% lower confidence limit ($BMDL_{10}$). The BMD_{10} and $BMDL_{10}$ for cancer and mutations were considered to be representative values to compare between the two endpoints in order to determine the relative magnitudes of the doses required to induce these endpoints. The $BMDL_{10}$, the lower limit on the dose corresponding to a predicted 10% response level, is commonly used as a point of departure in cancer assessments, and in the calculation of RfDs/RfCs for non-carcinogenic endpoints.

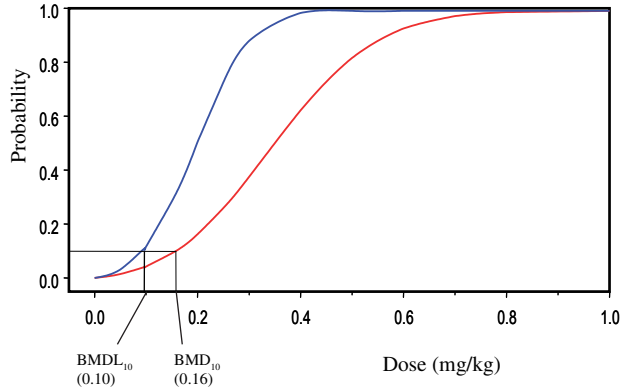
For the cancer incidence data, the multistage model was fitted using the BMD software available from the EPA (Gift, 2003). Additional risk above background risk was calculated by regressing the incidences of animals with tumors at each dose level against the administered dose. For the mutant frequency data, the induced quantal model of Kodell and West (1993) for continuous (pseudo-continuous) responses was fitted. Means and standard deviations of mutant frequencies at each dose level were modeled via a normal distribution, with a quadratic model for the mean, assuming an upper-tail background risk of 1%. The BMD_{10} calculated in this fashion corresponds to a dose that causes a shift in the mean of 1.1 standard deviations (Crump, 1995). Note that in the case of dichloroacetic acid (DCA), there were only three dose groups to fit three parameters.

CASE 1: CONSISTENT WITH A MUTAGENIC MODE-OF-ACTION

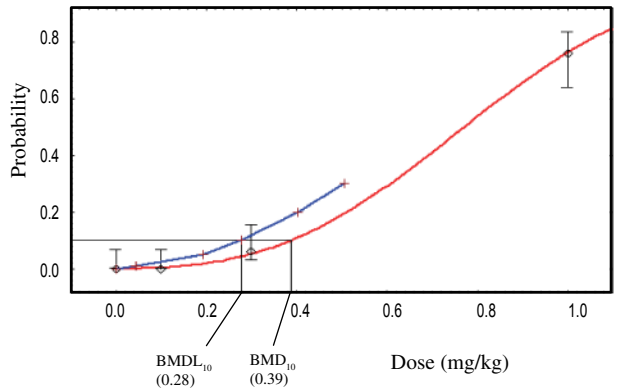
Riddelliine is a naturally occurring pyrrolizidine alkaloid that is a contaminant in human food (reviewed in Fu et al., 2004). Like other pyrrolizidine alkaloids, riddelliine is genotoxic in a number of short term *in vitro* and *in vivo* tests (reviewed in Fu et al., 2004). A recent National Toxicology Program (NTP) two-year carcinogenicity study found that riddelliine produces liver tumors in male and female rats (NTP, 2003; Chan et al., 2003). Using the Big Blue rat model, Mei et al., (2004) evaluated the mutagenicity of riddelliine in the *cII* transgene of rat liver. This study used the same treatment schedule, doses, and route of exposure as used in the NTP carcinogenicity study; however, in the mutagenicity study, the treatment was continued for only 12 weeks, after which time the animals were killed and DNA was extracted from their livers for mutational analysis.

In this analysis, the BMD for mutation induction is less than the BMD for tumor induction. This observation is consistent with a mutagenic MOA.

Dose Response for Liver Mutation Added Risk above Background Risk with 95% Upper Confidence Limit (Based on Normal Distribution with 1% Background Risk)



Dose Response for Liver Hemangiosarcoma Added Risk above Background Risk With 95% Upper Confidence Limit (Based on Multistage Model)

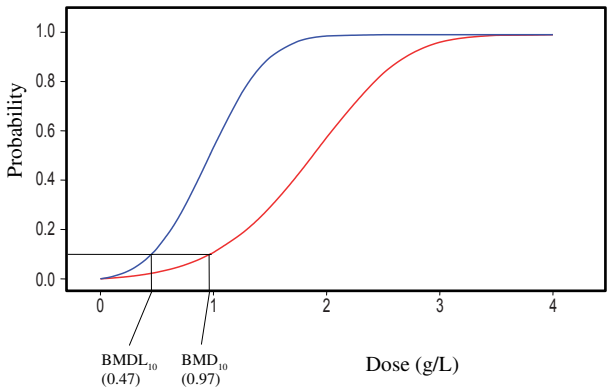


CASE 2: CONSISTENT WITH A NON-MUTAGENIC MODE-OF-ACTION

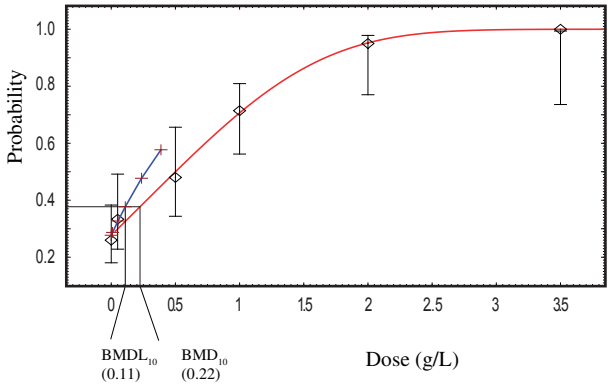
Dichloroacetic Acid (DCA) is a drinking water disinfection by-product. The genotoxicity data for DCA are reviewed in Moore et al. (2000). It is positive in the standard genotoxicity test battery. Using Tedlar bags to contain the vapor, it is positive in the Salmonella assay using strain TA100. It is also positive in the *in vitro* mammalian mouse lymphoma assay and the *in vivo* bone marrow micronucleus assay using a drinking water route of administration. The mutation data used for the BMD analysis came from Big Blue mice exposed to DCA *via* their drinking water for 60 weeks (Leavitt et al., 1997). We thank Dr. Anthony DeAngelo for providing the primary liver carcinoma data from his research published in DeAngelo et al. (1999).

In this analysis, the BMD for mutation induction is greater than the BMD for tumor induction. This observation is consistent with a non-mutagenic MOA.

Dose Response for Liver Mutation Added Risk above Background Risk with 95% Upper Confidence Limit (Based on Normal Distribution with 1% Background Risk)



Dose Response for Liver Carcinoma Added Risk above Background Risk with 95% Upper Confidence Limit (Based on Multistage Model)



SEEKING PARTNERS

To further the development and use of this approach to improve cancer risk assessment, NCTR has established a Cooperative Research and Development Agreement (CRADA) that will combine the genotoxicity expertise and laboratory capabilities of NCTR with the risk assessment and modeling expertise of *TERA* and ENVIRON. We invite companies, organizations and government agencies to join us by participating in and/or sponsoring studies investigating the best approach for experimentally obtaining and quantitatively analyzing *in vivo* mutagenicity data for use in cancer risk assessment.

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