Regulatory Toxicology and Pharmacology 65 (2013) 311–315

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

# A novel bottom-up approach to bounding low-dose human cancer risks from chemical exposures

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ARTICLE INFO

*Article history:*
Received 29 July 2012
Available online 23 January 2013

*Keywords:*
Bottom-up approach
Carcinogenic risk assessment
Unit risk
$q_1^*$
Upper 95% confidence bound risk estimate
DNA adducts
Formaldehyde

ABSTRACT

We propose a novel bottom-up approach to the bounding of low-dose human cancer risks from chemical exposures that does not rely at all upon high-dose data for human or animal cancers. This approach can thus be used to provide an independent "reality check" on low-dose risk estimates derived with dose-response models that are fit to high-dose cancer data. The approach (1) is consistent with the "additivity to background" concept, (2) yields central and upper-bound risk estimates that are linear at all doses, and (3) requires only information regarding background risk, background (endogenous) exposure, and the additional exogenous exposure of interest in order to be implemented. After describing the details of this bottom-up approach, we illustrate its application using formaldehyde as an example. Results indicate that recent top-down risk extrapolations from occupational cohort mortality data for workers exposed to formaldehyde are overly conservative by substantial margins.



## 1. Background

In 1976, Kenny Crump, David Hoel, Charles Langley, and Richard Peto published a landmark paper (Crump et al., 1976) showing that a non-decreasing dose–response relationship for cancer risk will be linear at sufficiently low doses as long as there is a non-zero background exposure to which the specific chemical exposures of interest simply add. This is the well-known "additivity to background" concept: at zero additional exposure, we are already somewhere up on the dose–response curve as a result of our non-zero background exposure, so the slope of the dose–response relationship at zero additional exposure will necessarily be non-zero and positive. Even a threshold dose–response relationship will have a non-zero slope at zero additional exposure if there are some individuals in the population of interest whose thresholds lie below their non-zero background exposure.

Then, in 1977, Crump, Harry Guess, and K.L. Deal published another landmark paper (Crump et al., 1977) that outlined the statistical and mathematical procedures for estimating and bounding the low-dose slope of the multistage dose–response model using constrained maximum likelihood methods and tumor data collected in laboratory animal bioassays conducted at very high exposure levels. It was in this paper that the now infamous "$q_1^*$", the upper 95% confidence bound on the coefficient of the linear term (i.e., the low-dose slope) of the presumed dose–response relationship, was created, and this value has dominated carcinogenic risk assessment ever since.

The dominance of $q_1^*$ in risk assessment has been a consequence of two factors. First, there is the tyranny of small numbers, i.e., the small numbers of animals that have been utilized in laboratory animal carcinogenicity studies, typically, only about 50 animals per sex per dose group. This number is so small that even if the observed tumor incidence in a treated group is zero (0/50), the exact binomial upper 95% confidence bound on the true response probability is 0.0582, so true risks up to this value cannot be confidently ruled out. It is also not possible to distinguish statistically at the $p = 0.05$ level between a response as high as 0.08 (4/50 tumor-bearing animals) in a treated group and a null response (0/50) in a control group using Fisher's exact test. If the goal of risk assessment is to bound the dose of a chemical that is associated with an upper bound incremental cancer risk of only one per million ($10^{-6}$), then one can conservatively "guesstimate" the required dose, using the low-dose linear hypothesis, as being about 100,000-fold lower than the highest dose that produces no significant increase, compared to controls, in the probability of developing cancer. This is common knowledge among biostatisticians, and a source of frustration and heartburn among many toxicologists; it is, nevertheless, an irrefutable "fact of life".

The second factor behind the dominance of $q_1^*$ is that until recently, the background exposures that may be responsible, at least in part, for our background cancer risks have not been quantified (two notable exceptions are radiation and our background body

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http://dx.doi.org/10.1016/j.yrtph.2013.01.004

burdens of dioxin-like compounds). Generally, little attention has been focused on quantifying background chemical exposures, and the exposures of interest have routinely been expressed as increments above whatever the background exposures might be. This is primarily due to the fact that human background exposures are complicated, uncontrolled, and usually unmeasured, while the animal studies that attempt to carefully control and minimize these background exposures have not routinely included measures of the corresponding internal (endogenous) doses that can arise via normal metabolism and other internal biochemical reactions.

Without knowing what background exposure is, expressed preferably as the concentration of a relevant exposure biomarker, e.g., DNA adducts, in the target tissue of interest, the only way to estimate the slope of the dose–response relationship at low doses has been via downward extrapolation from the observed tumor responses in small numbers of animals (or occupationally exposed people) at high external exposure levels, which forces us into the $q_1^*$conundrum. However, this situation has changed recently, and the change could profoundly alter carcinogenic risk assessment going forward, at least for those potentially carcinogenic substances that are always present in our bodies, even absent external exposure, because they are produced continuously by normal biochemical processes such as metabolism and biochemical synthesis and degradation. The key technological advance underpinning our novel "bottom-up" approach to risk assessment is the extraordinary ability to distinguish between and separately quantify the relevant internal exposures in target tissues that arise from internal background (endogenous) and external (exogenous) sources. In what follows, we outline this alternative approach to estimating and bounding low-dose cancer risks for such substances, and illustrate the potential for its application with the specific example of formaldehyde, an important commodity chemical that is currently under review by the US Environmental Protection Agency (USEPA).

## 2. The bottom-up approach

Let $P_0$ represent the background lifetime risk of a tissue-specific cancer in people, such as nasopharyngeal cancer or leukemia. Let $C_0$ represent the mean tissue-specific background steady-state concentration of a biomarker, such as a specific DNA adduct, that is presumed to be causally related to these cancers. Then the ratio $P_0/C_0$ provides an estimate of the low-dose slope of the relationship between the cancer risk and the corresponding tissue-specific DNA adduct concentration. Similarly, if $C_{0L}$ represents the *lower* 95% confidence bound estimate for the same background adduct concentration, then the ratio $P_0/C_{0L}$ provides an *upper* 95% confidence bound on the low-dose slope. This latter ratio is thus directly comparable to the $q_1^*$ derived from high dose animal studies, as well as the upper bound slope estimates for the low-dose linear dose–response relationships that are typically inferred from epidemiologic analyses of occupational cohort cancer mortality, provided only that the dose metrics used in these two kinds of studies (animal bioassays and cohort mortality studies) are converted into the corresponding equivalent tissue-specific adduct concentrations.

The key elements of this bottom-up approach are illustrated in Fig. 1. What is most important to appreciate is that the central and upper bound slope estimates derived using this approach do not depend in any way on high-dose carcinogenicity data for laboratory animals or humans. The approach thus provides a completely independent "reality check" on low-dose slope estimates like $q_1^*$ that are derived from analyses of high-dose laboratory animal tumor incidence data or occupational cancer mortality data.

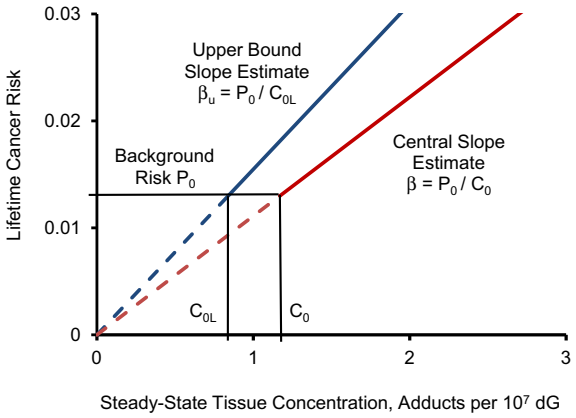


**Fig. 1.** Illustrating the "bottom-up" approach to bounding additional human cancer risks that may be associated with low level chemical exposures. $P_0$ is the background lifetime risk of a tissue-specific cancer. $C_0$ and $C_{0L}$ are the central and lower 95% confidence bound estimates of the steady-state background concentration of specific DNA adducts linked to the cancer in the same tissue. $\beta$ and $\beta_u$ are the bottom-up central and upper 95% confidence bound estimates of the low-dose slope of the cancer risk-DNA adduct relationship.

## 3. An illustration of the bottom-up approach using currently available data for formaldehyde

Formaldehyde is a highly reactive chemical and an essential metabolic intermediate that is generated endogenously in all living cells, and $N^2$-hydroxymethyl-deoxyguanosine (dG) adducts have been detected and quantified in various tissues of rats (Lu et al., 2010 and 2011) and cynomolgus macaques (Moeller et al., 2011) exposed to various concentrations of stable isotope-labelled [$^{13}CD_2$]-formaldehyde by inhalation. These formaldehyde-DNA adducts are potentially promutagenic because adduction takes place on the amino groups participating in Watson–Crick base pairing, and adduct formation is widely considered to be a key event in the initiation of mutations that lead to carcinogenesis (Swenberg et al., 2011). Thus, the tissue-specific concentration of these adducts provides an excellent internal dose metric with which to illustrate the bottom-up approach to bounding the low-dose slope of dose–response relationships for human cancer risk.

The use of [$^{13}CD_2$]-formaldehyde permits the simultaneous measurement of both endogenous and exogenous formaldehyde-DNA adducts with sensitive Liquid Chromatography–Electrospray Ionization-Tandem Mass Spectrometry-Selected Reaction Monitoring (LC–ESI-MS/MS-SRM) methods. While endogenous dG adducts were detected in all of the examined tissues, exogenous dG adducts formed with inhaled [$^{13}CD_2$]-formaldehyde were detected only in the tissues taken from the site of initial contact with exogenous formaldehyde, i.e., rat and monkey nasal respiratory epithelium (Swenberg et al., 2011).

Because no exogenous dG adducts were detected in these studies in any distant site tissues, including bone marrow and the blood, we can state with confidence that if such exogenous adducts were present in these tissues, then their amounts would necessarily have been smaller than the LC–ESI-MS/MS-SRM method's detection limit (DL). We have therefore used the method's DL (reported in Moeller et al. (2011) as $20 \times 10^{-18}$ mol) as a worst case upper bound on the level of exogenous dG adducts that could be present and yet remain undetected in the bone marrow of [$^{13}CD_2$]-formaldehyde-exposed monkeys. The above molar DL was converted to an equivalent DL expressed in terms of the number of adducts, namely, $1.03 \times 10^{-3}$ per $10^7$dG, using the average amount of monkey DNA collected in the bone marrow samples (Moeller et al., 2011), and the amount of guanine (0.20, expressed as a fraction) that is present in monkey DNA (Casanova et al., 1991).