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Comparison of cancer risk estimates based on a variety of risk assessment methodologies

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Abstract

The EPA guidelines recommend a benchmark dose as a point of departure (PoD) for low-dose cancer risk assessment. Generally the PoD is the lower 95% confidence limit on the dose estimated to produce an extra lifetime cancer risk of 10% (LTD_{10}). Due to the relatively narrow range of doses in two-year bioassays and the limited range of statistically significant tumor incidence rates, the estimate of the LTD_{10} is constrained to a relatively narrow range of values. Because of this constraint, simple, quick estimates of the LTD_{10} can be readily obtained for hundreds of rodent carcinogens from the Carcinogenic Potency Database (CPDB) of Gold et al. Three estimation procedures for LTD_{10} are described, using increasing information from the CPDB: (A) based on only the maximum tolerated dose (the highest dose tested); (B) based on the TD_{50}; and (C) based on the TD_{50} and its lower 99% confidence limit. As expected, results indicate overall similarity of the LTD_{10} estimates and the value of using additional information. For Method (C) the estimator based on the $[\{(TD_{50})^{0.36} \times (LoConf)^{0.64}\}/6.6]$ is generally similar to the estimator based on the one-hit model or multistage model LTD_{10}. This simple estimate of the LTD_{10} is applicable for both linear and curved dose responses with high or low background tumor rates, and whether the confidence limits on the TD_{50} are wide or tight. The EPA guidelines provide for a margin of exposure approach if data are sufficient to support a nonlinear dose–response. The reference dose for cancer for a nonlinear dose–response curve based on a 10,000-fold uncertainty (safety) factor from the LTD_{10}, i.e., the $LTD_{10}/10{,}000$, is mathematically equivalent to the value for a linear extrapolation from the LTD_{10} to the dose corresponding to a cancer risk of $<10^{-5}$ ($LTD_{10}/10{,}000$). The cancer risk at $<10^{-5}$ obtained by using the q_1^* from the multistage model, is similar to $LTD_{10}/10{,}000$. For a nonlinear case, an uncertainty factor of less than 10,000 is likely to be used, which would result in a higher (less stringent) acceptable exposure level.
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1. Introduction

In the U.S. Environmental Protection Agency *Proposed Guidelines for Carcinogen Risk Assessment* (1996, 1999), the mode of action of a chemical is evaluated for use in the cancer risk assessment. The basic default is to assume linearity of the dose response if that is suggested by the mode of action analysis or if the mode of action is not understood. The mode of action may lead to a nonlinear dose response or may theoretically have a threshold, and then a margin of exposure (MOE) analysis and an uncertainty factor approach would be applicable. The MOE analysis would consider more than tumors, generally the toxicologic events that can lead to tumor development for a given chemical.

The EPA carcinogen risk assessment guidelines (1996) select a benchmark dose (BMD) approach for low-dose cancer risk assessment. The BMD generally is the dose associated with an extra lifetime tumor risk of 10% (TD_{10}). This level of risk was selected because it is about as low as most experiments permit direct estimation of statistically significant increases in tumor incidence without reliance on model selection, fitting, and extrapolation outside the observable experimental data range. If the data for a chemical warrant, a lower level of risk may be selected for the BMD. In order to account for experimental variability, a lower 95% confidence

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limit on the TD_{10}, designated by LTD_{10}, generally is used as a point of departure (PoD) for low-dose cancer risk assessment. If the available information supports a nonlinear dose response below the PoD, an MOE is calculated between the PoD, generally the LTD_{10}, and anticipated human doses. If a nonlinear dose response below the PoD is not supportable, linear extrapolation from the PoD to zero is employed. In either case, low-dose cancer risk assessment is based on the PoD, which generally is the LTD_{10}. Thus, estimation procedures for the LTD_{10} are required. Since human data rarely provide adequate quantitative estimates of cancer risk as a function of dose, the LTD_{10} usually is estimated from animal bioassay data.

When estimating "safe" dose levels for humans based on the PoD, it is necessary to take into account the uncertainty in extrapolating results from animals to humans. The uncertainty factor for this extrapolation (U_A) usually is set equal to a default value of 10 unless information is available about the relative sensitivity of the test species and humans. That is, this uncertainty factor allows for the possibility that an average human may experience the same adverse health effect at a dose that is a factor of U_A lower than the animal dose. Another uncertainty factor (U_H) is used to allow for inter-individual variability among humans in sensitivity. Again, a default value of 10 is used for U_H unless information is available about inter-individual sensitivity. Since the LTD_{10} represents a 10% risk level, an uncertainty factor (U_L) may be introduced to consider a lower effect level. Renwick (1995) and Schwartz (1995) suggest the use of an additional safety factor due to the severity of cancer. The Food Quality Protection Act (U.S. Congress Committee on Agriculture, 1996) requires "consideration" of an additional uncertainty factor (U_C) of up to 10 to account for potential additional sensitivity of children. Hence, a total default uncertainty factor, or MOE, of $U = U_A \times U_H \times U_L \times U_C = 10^4 = 10{,}000$ could result. Since it is unlikely that all uncertainties need to be at their default values simultaneously, in order to reduce unnecessarily compounding uncertainty, the EPA (1991) has suggested replacing 10,000 by 3000 for this case.

Rodent cancer tests are designed to maximize the probability of detecting chemical carcinogens from lifetime exposures with relatively small numbers of test animals, generally about 50 animals in each of two or three doses plus controls in both sexes of one or more species. The maximum tolerated dose (MTD), MTD/2, and recently the MTD/4 are the standard dose levels used in bioassays of the National Toxicology Program. This experimental design, with a narrow range of doses, was not intended to provide information to quantitatively assess the risk to humans from chemical exposures at low doses, but often this is the only information available. Several authors have discussed the limited amount of information on low-dose cancer risk that is provided by the typical bioassay (Ames and Gold, 2000; Bernstein et al., 1985; Gold et al., 1998). Bernstein et al. (1985) showed for a bioassay with two dose groups plus controls that estimates of potency, obtained from a statistically significant bioassay as measured by the TD_{50}, are constrained to a narrow range of 32-fold about the maximum dose tested, in the absence of tumors in all dosed animals (which rarely occurs) in the high dose group. Therefore, risk estimates are constrained by the bioassay design.

Gaylor and Gold (1995) exploited this constraint to obtain a quick estimate of potency for carcinogens based only on the MTD obtained from a 90-day study without conducting a lifetime bioassay. Based on a study of the outcomes of 139 rodent carcinogens identified by the National Toxicology Program (NTP), Gaylor and Gold (1998) show that the MTD/7 is likely to be within a factor of 10 of the LTD_{10}. Since estimates of cancer potency from replicated two-year bioassays have been shown to vary by a factor of 4 around a median value (Gaylor et al., 1993), there may be little gain in the precision of cancer risk estimates derived from a two-year bioassay compared to the estimate based on the MTD from only a 90-day study.

Since EPA regulatory policy calls for use of the LTD_{10} from rodent bioassays, this paper investigates how the estimate of LTD_{10} can be improved over the MTD/7 by using additional information available on rodent bioassays in the Carcinogenic Potency Database. A still better estimate of the LTD_{10} can be obtained if both the TD_{50} and a lower confidence limit for the TD_{50} are available, as is the case for bioassays in the CPDB. All of these estimates of LTD_{10} are constrained by the bioassay design and the rarity in bioassay results of 100% tumor incidence in the target organ.

Further, with respect to risk estimation, the analyses presented in this paper indicate the similarity between a "safe" dose for a cancer risk of $<10^{-5}$ based on linear extrapolation from the LTD_{10} vs. a "safe" dose for a nonlinear dose response curve using default uncertainty factors. The two approaches give the same regulatory safe dose level if the total uncertainty factor is 10,000; for a nonlinear dose response, the total uncertainty factor will usually be less than 10,000, and the "safe" dose level will be higher (see chloroform example below). Additionally, for a nonlinear dose response, the risk assessment may use a noncancer endpoint as the PoD for a rodent carcinogen if it occurs at a lower dose and by a similar mode of action, e.g. hepatotoxicity in dogs for chloroform. The 1999 EPA guidelines state: "If, in a particular case, the evidence indicates a threshold, as in the case of carcinogenicity being secondary to another toxicity that has a threshold, the margin of exposure analysis for the toxicity is the same as is done for a noncancer endpoint, and an RfD or RfC

for that toxicity also may be estimated and considered in cancer assessment." (U.S. Environmental Protection Agency, 1999)

2. Methods

The data used from the CPDB were from two sources and were selected using the same criteria as our earlier work on MTD and the benchmark dose (Gaylor and Gold, 1995, 1998): (i) NCI/NTP chronic bioassays that were evaluated as "positive" in NCI studies prior to 1979, or having "clear" or "some" evidence of carcinogenicity in later studies, and (ii) non-NCI/NTP (literature) bioassays which were considered positive by the authors of the study and exhibited a statistically significant positive dose response of $P < 0.05$ (two-tailed). Experiments were considered only if the chemical was administered for at least 18 months with the termination of the study after 21 months. Three methods for estimating the LTD_{10} are described, which utilize increasing information from the CPDB about rodent bioassay results. Method A uses only the MTD; Method B uses only the TD_{50}; Method C uses the TD_{50} and its lower 99% confidence limit. The LTD_{10} is a measure of the lower 95% confidence limit on TD_{10}, whereas the CPDB reports the lower 99% confidence limit on TD_{50}. Appendix A describes the calculation of the 95% LTD_{10} based on the results in the CPDB. This calculation is used in Methods B and C.

2.1. Method A

As discussed in Section 1, if only the MTD is available, a simple, quick estimate of the LTD_{10} is provided by the MTD/7 (Gaylor and Gold, 1998), designated here as Method A:

$$LTD_{10} = MTD/7. \tag{1}$$

2.2. Method B

Method B uses only the TD_{50} values from the CPDB (Gold et al., 1999, http://potency.berkeley.edu; Gold and Zeiger, 1997). The database includes positive results for 2041 experiments in which the test agent was evaluated as carcinogenic by either the National Cancer Institute/National Toxicology Program (NCI/NTP) (534 experiments) or the published authors from the general literature (1507 experiments). For the most potent (lowest) TD_{50} value from each positive experiment, the average ratio of the lower 99% confidence limit (LoConf) to the TD_{50} is 0.504. That is, on the average, $LoConf = 0.504 \times TD_{50}$. Substituting this result into Eq. (A.9) from Appendix A gives on the average

$$\begin{aligned} LTD_{10} &= (TD_{50})^{0.36} \times (0.504 \times TD_{50})^{0.64}/6.6 \\ &= TD_{50}/10.2. \end{aligned} \tag{2}$$

Thus, a simple, quick estimate of the LTD_{10} can be obtained from just the TD_{50} values in the CPDB by using the $TD_{50}/10.2$, designated here as Method B.

2.3. Method C

Both the TD_{50} and the two-sided 99% confidence limits (LoConf, UpConf) are reported in the CPDB (Gold et al., 1999, http://potency.berkeley.edu; Gold and Zeiger, 1997) from fitting the one-hit model to experimental dose–response data. The one-hit model was selected because it is the most conservative of the multistage models, and upper confidence limits on risk estimates from bioassays also tend toward linearity at low doses. When both the TD_{50} and the two-sided 99% confidence limits are available, it is shown in Appendix A for the one-hit model that the lower 95% confidence limit for the TD_{10}, i.e., LTD_{10} is

$$LTD_{10} = \left[(TD_{50})^{0.36} \times (LoConf)^{0.64}\right] \Big/ 6.6 \tag{3}$$

designated here as Method C. Since the one-hit model is the most conservative form of the family of multistage models of carcinogenesis, the above estimate of the LTD_{10} provides at least 95% confidence that the true value of the TD_{10} is above this lower confidence limit for this family of models.

Method C can be validated by fitting the one-hit model and the multistage model to tumor incidence data, calculating the LTD_{10} directly from the fitted model, and comparing these results to the values obtained by Method C from Eq. (3). Cases for comparisons were selected from the CPDB to cover conditions that affect the value of the LTD_{10} relative to the TD_{50}. Only cases with four or more dose groups, including the controls, were selected because this allows more variation and is therefore a more powerful test of the difference between methods. The conditions covered include linear and curvilinear dose–response curves with low tumor incidence (less than 3%) in the control animals and moderate to high tumor incidence (greater than 7%) in the controls. Also, for the above conditions, one case was selected where the LoConf was within a factor of 1.5 of the TD_{50} (tight confidence limit) and one case was selected where the LoConf was a factor of 2 or more below the TD_{50} (wide confidence limit). The results are summarized in Table 1.

3. Results

For the cases selected for Table 1, Method C (Eq. (3)), which uses the TD_{50} and LoConf, gave estimates

Table 1
Calculation of the LTD_{10} by Method C and directly by fitting the one-hit and multistage models to the tumor incidence data

Case number[a]	Dose–response	Control incidence	Confidence limits[b]	Tumor incidence	LTD_{10} (mg/kg/day)		
					Method C	One-hit model	Multistage model
4 doses (including controls)							
1	Linear	<3%	Tight	0/50, 6/48, 26/53, 21/53	45	34	44
2	Linear	<3%	Wide	0/18, 0/20, 1/19, 9/18	2.9	2.8	3.4
3	Linear	>7%	Tight	39/297, 24/90, 32/87, 136/148	0.081	0.047	0.047
4	Linear	>7%	Wide	7/80, 5/69, 4/80, 15/66	12	11	14
5	Curved	<3%	Tight	1/87, 29/90, 58/90, 63/82	1.7	1.7	5
6	Curved	<3%	Wide	0/40, 0/48, 0/50, 8/48	80	61	71
—	Curved	>7%	Tight	No cases			
—	Curved	>7%	Wide	No cases			
>4 doses (including controls)							
7	Linear	<3%	Tight	1/40, 0/39, 16/38, 38/39, 37/38	0.0066	0.0057	0.0057
8	Linear	<3%	Wide	0/40, 3/16, 1/16, 3/20, 5/20	17	16	16
9	Linear	>7%	Tight	10/125, 54/119, 43/95, 31/71, 37/72, 51/69, 56/72	2.5	1.3	1.3
10	Linear	>7%	Wide	8/26, 12/26, 5/29, 11/28, 10/32, 15/27, 17/27	2.7	2.3	2.3
11	Curved	<3%	Tight	0/50, 0/50, 0/50, 0/50, 10/50, 50/50	73	70	307
12	Curved	<3%	Wide	1/384, 1/108, 0/66, 0/35, 6/33	8.4	8.3	5.8
13	Curved	>7%	Tight[c]	4/20, 6/39, 2/20, 11/20, 19/27	0.31	0.22	0.60
—	Curved	>7%	Wide	No cases			

[a] Line number and description of data from the plot in Gold et al. (1997). Identification of experimental results: 1—acetaldehyde, FR, Wistar, nose adenocarcinoma (line 5); 2—acrylonitrile, MR, Charles River CD1, Zymbal's gland squamous cell carcinoma (line 145); 3—dieldrin, FM, CF-1, liver tumors (line 1696a); 4—acifluorfen, FM, Charles River CD1, liver tumors (line 129); 5—2-acetylaminofluorene, FM, BALB/cStCrlfC3Hf/Nctr, urinary bladder tumors (line 87a); 6—Diftalone, MM, BALB/cLacDp, liver angiosarcoma (line 1769); 7—nitrosoethylurethane, FR, F344/DuCrj, upper gastrointestinal tract tumors (line 3684); 8—*N*-nitrosodiethanolamine, FR, Fischer 344, liver cholangiocarcinoma (line 3624c); 9—benzindine.2HCl, FM, C57BL/6JfC3Hf/Nctr × BALB/cStCrlfC3Hf/Nctr inter se, hepatocellular adenocarcinoma (line 533); 10—3,3′-dimethylbenzidine.2HCl, MM, BALB/cStCrlfC3Hf/Nctr, lung tumors (line 1883); 11—butylated hydroxyanisole, MR, F344/DuCrj, forestomach tumors (line 790); 12—2-acetylaminofluorene, FM, BALB/cStCrlfC3Hf/Nctr, urinary bladder transitional-cell carcinoma (line 84a); 13—*N*-nitrosodiethanolamine, MR, Fischer 344, liver tumors (line 3628).

[b] Tight limits: $TD_{50}/LoConf < 1.5$. Wide limits: $TD_{50}/LoConf > 2$.

[c] $(TD_{50}/LoConf) = 1.65$.

within a factor of 2 of the LTD_{10} calculated from fitting the one-hit model to the tumor incidence data. This result is consistent for both linear and upward curved dose–responses with high or low tumor rates in the controls and tight ($TD_{50}/LoConf < 1.5$) or wide ($TD_{50}/LoConf > 2$) confidence limits for the TD_{50} (Table 1). As also noted by Gaylor (1992) for a database of 143 dose–response curves from chronic bioassays, there are very few cases of upward curvature in the dose response when the incidence of tumors is high in the controls; therefore, as expected, there are no cases for some categories with high background tumor rates in Table 1.

It was observed in the CPDB that the TD_{50} tends to be somewhat less than the geometric mean of the LoConf and UpConf. Hence, the above approximation for the LTD_{10} in Eq. (3) tends to be conservative (low). Further, the one-hit model is the most conservative form of the family of multistage models of carcinogenesis. Therefore, the above approximation for the LTD_{10} generally provides at least 95% confidence that the true value of the TD_{10} is above this lower confidence limit.

The values of the LTD_{10} calculated by Method C for the cases in Table 1 varied about the values of the LTD_{10} estimated by fitting the multistage model to the tumor incidence data. The LTD_{10} calculated by Method C was similar to the LTD_{10} estimated with the multistage model, and the differences were within a factor of 5. An order of magnitude span of uncertainty has been estimated for setting reference doses for noncancer endpoints, i.e., the estimated reference dose (RfD) might be three times higher or three times lower than the true value (Barnes and Dourson, 1988). The RfD is defined as an estimate (spanning perhaps an order of magnitude) of the daily dose for the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious health effects during a lifetime (Barnes and Dourson, 1988).

Table 2 compares the estimates of LTD_{10} for Methods A, B, and C and demonstrates both the overall similarity of estimates and the value of using additional information to estimate LTD_{10}, regardless of the route of administration in a bioassay. For the NCI/NTP studies the MTD was based on 90-day studies. For studies cited in the general literature, the maximum dose tested was assumed to approximate the MTD. In keeping with generally conservative regulatory policy, the TD_{50} for a chemical was selected as the most potent site (lowest) significant TD_{50}. Method A, using just the MTD, has more variation and also varies more from Method C than does Method B. Methods B and C are similar to each other.

When a nonlinear dose–response curve in the low-dose range can be justified, an MOE between the LTD_{10} and the anticipated human exposure level is considered for cancer risk assessment (U.S. Environmental Protection Agency, 1996). Presumably, an acceptable MOE should be as large as the uncertainty factor used in setting an RfD or greater than 10,000 for a linear dose response (10^{-5} risk).

In all cases, the LTD_{10} is the starting point for cancer risk assessment. A summary is given in Table 3 of the three quick methods presented for estimating the LTD_{10} from the CPDB. A comparison of doses for a lifetime cancer risk of $<10^{-5}$ obtained by employing these three methods is made with the dose obtained by linear extrapolation from the LTD_{10} obtained by fitting the multistage model. Division of the LTD_{10} (which corresponds to a risk of 0.1 or less) by 10,000 results in a risk of less than 10^{-5}, i.e., $0.1/10^{-4} = 10^{-5}$. The comparisons of the procedures relative to the MTD in the last column of Table 3 are based on average or median relationships for animal carcinogens in the CPDB as described in the footnotes to the table. On average, all estimates are within a factor of 2 of each other if the 10,000-fold uncertainty factor is used for the nonlinear case. Methods B and C are most similar and are somewhat more stringent than the multistage model, in part because the TD_{50} is based on the conservative one-hit model. Obviously, when "safe" doses for the linear case and nonlinear case are both based on the LTD_{10}/10,000, the resulting "safe" doses are mathematically identical. If a less stringent overall uncertainty factor, e.g., 3000 were to be selected for a nonlinear dose response, then the estimated "safe" dose would be 3.3-fold higher.

4. Example: Chloroform

For the cancer risk assessment of chloroform, the EPA used kidney tumors in male Osborne-Mendel rats in a study by Jorgenson et al. (1985). As reported in the CPDB, the incidences were 5/301, 6/313, 7/148, 3/48, and 7/50 at human equivalent doses of 0, 2.9, 5.9, 13.2, and 26.5 mg/kg body weight (bw)/day of chloroform in the drinking water, respectively, assuming water consumption of 25 ml/day for the male rats. Human equivalent doses are obtained by adjusting doses on the basis of milligram of chloroform per body weight to the 3/4-power. Using a standard body weight of 70 kg for humans and the CPDB standard 0.5 kg for male rats, results in a 3.4-fold reduction in the doses administered to rats in order to obtain the human equivalent doses. Fitting the quantal-linear (one-hit) model to these tumor

Table 2
Ratio of the LTD_{10} for Methods A and B to the LTD_{10} for Method C

Route	N[a]	Mean	Median	SD[b]	Range	90% Range	Correlation[c]
Method A/Method C							
Inhalation	30	4.89	1.10	8.92	0.10–41.8	0.18–20.6	0.879
Oral	371	3.52	1.81	5.97	0.10–63.2	0.30–13.2	0.919
All routes	399	3.65	1.81	6.24	0.10–63.2	0.30–15.4	0.919
Method B/Method C							
Inhalation	30	0.99	0.95	0.19	0.78–1.63	0.79–1.38	0.998
Oral	371	0.98	0.95	0.16	0.71–2.07	0.80–1.27	0.999
All routes	399	0.98	0.95	0.16	0.71–2.07	0.80–1.27	0.999

[a] Number of cases.
[b] Standard deviation.
[c] Pearson correlation coefficient for $\log LTD_{10}$.

Table 3
Comparison of quick estimates of cancer risk that are readily obtainable from CPDB results

Procedure	Estimated "Safe" dose	Relative to the MTD[a]
(I) *Historical risk assessment based on q_1^* and the linearized multistage model for a cancer risk less than* 10^{-5}	$10^{-5}/q_1^*$	MTD/74,000[b]
(II) *New EPA guidelines using LTD_{10}*		
(A) Linear extrapolation from the LTD_{10}[c] for a cancer risk $<10^{-5} = LTD_{10}/10{,}000$		
Method A	MTD/70,000	MTD/70,000
Method B	$TD_{50}/102{,}000$	MTD/134,000[d]
Method C	$(TD_{50})^{0.36}(LoConf)^{0.64}/66{,}000$	MTD/127,000[e]
(B) Nonlinear dose–response curve reference dose (RfD)[f]:		
(1) $RfD = LTD_{10}/(\text{uncertainty factor}) = LTD_{10}/10{,}000$[g]		
Method A	MTD/70,000	MTD/70,000
Method B	$TD_{50}/102{,}000$	MTD/134,000
Method C	$(TD_{50})^{0.36}(LoConf)^{0.64}/66{,}000$	MTD/127,000
(2) LTD_{10}/uncertainty factor of 3000 (nonlinear case)[h]		
Method A	MTD/21,000	MTD/21,000
Method B	$TD_{50}/30{,}600$	MTD/40,000
Method C	$(TD_{50})^{0.36}(LoConf)^{0.64}/19{,}800$	MTD/38,000

[a] MTD is the maximum tolerated dose for NCI/NTP studies and the maximum tested dose for the general literature studies.
[b] Gaylor and Gold (1995).
[c] LTD_{10} is a lower 95% confidence limit on the dose estimated to produce an extra lifetime cancer risk of 10%.
[d] From Table 2 the median value of the ratio of the LTD_{10} for Method B to Method C is 0.95, giving 127,000/0.95 = 134,000.
[e] From Table 2 the median value of the ratio of the LTD_{10} for Method A to Method C is 1.81, giving $70{,}000 \times 1.81 = 127{,}000$.
[f] RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of the daily dose for the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious health effects during a lifetime (Barnes and Dourson, 1988).
[g] Multiple of four default uncertainty factors of 10 each ($10^4 = 10{,}000$) for extrapolation from results in animals to humans, inter-individual sensitivity, extrapolation from an effect level (LTD_{10}), and possible extra sensitivity of children per the Food Quality Protection Act of 1996 (U.S. Congress Committee on Agriculture, 1996).
[h] Reduction of any one of the uncertainty factors from 10 to 3 giving a total uncertainty factor of $3 \times 10^3 = 3000$; or as suggested by the EPA (1991) use a maximum uncertainty factor of 3000 for the product of four uncertainty factors to reduce compounding conservatism.

incidence data using the EPA Benchmark Dose Software (BMDS) gives the $LTD_{10} = 15$ mg/kg bw/day. The LTD_{10} listed in the EPA IRIS database (U.S. Environmental Protection Agency, 2001, 2002) of 23 mg/kg bw/day differs from the CPDB estimate primarily because of differences in the calculation of daily water consumption per kg bw for the rats.

Using the three methods described in this paper, quick estimates of the LTD_{10} for chloroform can be obtained from the following values listed in the CPDB: $TD_{50} = 519$, LoConf = 265, and MTD = 90 mg/kg bw/day, which converted to human equivalent doses on the basis of $(bw)^{3/4}$ are: $TD_{50} = 519/3.4 = 153$, LoConf = 265/3.4 = 78, and MTD = 90/3.4 = 26.5 mg/kg bw/day.

Method A: $LTD_{10} = MTD/7 = 26.5/7 =$ 3.8 mg/kg bw/day.

Method B: $LTD_{10} = TD_{50}/10.2 = 153/10.2 =$ 15 mg/kg bw/day.

Method C: $LTD_{10} = (TD_{50})^{0.36}(LoConf)^{0.64}/6.6 = (153)^{0.36}(78)^{0.64}/6.6 = 15$ mg/kg bw/day.

In this case, both Methods B and C gave exactly the same estimate of the $LTD_{10} = 15$ mg/kg bw/day, as did fitting the one-hit model to the tumor incidence data.

The EPA, in its risk assessment, indicated that chloroform was not expected to produce rodent tumors by a mutagenic mode of action and that "available evidence indicates that chloroform-induced carcinogenicity is secondary to cytotoxicity and regenerative hyperplasia; hence, the Agency relies on a nonlinear dose–response approach and the use of a margin-of-exposure analysis for cancer risk. The Agency has also chosen not to rely on a mathematical model to estimate a point of departure for cancer risk estimate, because the mode of action indicates that cytotoxicity is the critical effect and the reference dose value is considered protective for this effect." (U.S. Environmental Protection Agency, 2002)

For the MOE analysis, the EPA compared the cancer PoD (23 mg/kg/day in rats) to an RfD of 0.01 mg/kg/day based on hepatotoxicity in dogs, the most sensitive species (Heywood et al., 1979). This led to an "MOE of 2000, which is considered large. Thus, in this case, the RfD for noncancer effect is also considered adequately protective of public health for cancer effects by the oral route, on the basis of the nonlinear dose response for chloroform and the mode of action for both cancer and noncancer effects having a common link through cytotoxicity." (U.S. Environmental Protection Agency, 2002).

If the MOE were based on the 15 mg/kg/day PoD for cancer from the CPDB using Method C, the resulting

ratio would be 1500, which is also large and presumably protective for cancer.

The EPA concluded, "Under the Proposed Guidelines for Carcinogen Risk Assessment, chloroform is *likely to be carcinogenic to humans by all routes of exposure* under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues. Chloroform is *not likely to be carcinogenic to humans by any route of exposure* under exposure conditions that do not cause cytotoxicity and cell regeneration." (U.S. Environmental Protection Agency, 2002)

5. Discussion and conclusions

Gaylor and Gold (1998) show that an estimate of the LTD_{10} can be obtained for animal carcinogens from the MTD determined for a 90-day study without conducting a chronic exposure study. They noted for 139 NCI/NTP animal carcinogens that the MTD/7 (Method A) was generally within a factor of 10 of the LTD_{10} obtained from chronic studies. This provides a quick preliminary estimate for the LTD_{10} when data are not available from a chronic exposure bioassay. However, deviations greater than a factor of 10 between the MTD/7 and the LTD_{10} occasionally occur for exposures of less than two years or where several doses were tested and results from one or more of the high doses were deleted due to a decline in tumor incidence. Conversely, values of the MTD/7 less than one-tenth of the LTD_{10} occasionally have been noted for cases with a low tumor incidence with borderline statistical significance. A better estimate of the LTD_{10} can be obtained when the TD_{50} is available from a lifetime bioassay by using the $TD_{50}/10.2$ (Method B). With additional information for both the TD_{50} and its lower confidence limit reported in the CPDB, Method C (Eq. (3)) gave similar estimates to the LTD_{10} obtained by fitting the multistage model to the tumor incidence data for both linear and curved dose responses with high or low tumor rates in the controls and tight or wide confidence limits for the TD_{50}. Thus, relatively precise estimates of the LTD_{10} can be readily calculated from the TD_{50} and LoConf reported in the CPDB. Because of the similarity in results for Methods B and C, estimates of "safe" doses for animal carcinogens based on the LTD_{10} or TD_{50} will give similar results. Further, linear extrapolation to the dose corresponding to a lifetime cancer risk of $<10^{-5}$ and the reference dose calculated for a nonlinear dose–response curve employing a total uncertainty factor of 10,000 (10-fold each for animal to human extrapolation, inter-individual variability, extrapolation from an effect level $\{LTD_{10}\}$, and sensitivity for children) are mathematically identical ($LTD_{10}/10{,}000$).

The default value of 10,000 for the uncertainty factor may not be used for a chemical. For a nonlinear case, it has been suggested that compounding conservatism can be reduced by limiting the total uncertainty factor to a maximum of 3000 when four uncertainty factors are involved (U.S. Environmental Protection Agency, 1991), and this would result in a "safe" dose that is 3.3-fold higher. For chloroform, a nonlinear case, the EPA (2002) has used an uncertainty factor of 1000. The 10-fold uncertainty factor for extrapolation from rodent data to humans may also be lowered under some conditions, such as when humans may be less sensitive when a physiologically based pharmacokinetic (PBPK) model is used. If a surface area correction is used to estimate the cancer LTD_{10}, then it would be appropriate to limit the interspecies uncertainty factor to 3 instead of 10, since interspecies scaling has already taken place. This would give a "safe" dose estimate that is 3.3-fold higher. Historically, cancer risk estimation has made an interspecies scaling factor adjustment using body surface area, whereas the RfD for noncancer endpoints has used mg/kg body weight/day. Some consideration to harmonize these different practices seems reasonable under the new Guidelines. Sometimes sufficient mechanistic data are available to allow for a reduction in the uncertainties about interspecies extrapolation. The EPA, for example, has utilized toxicokinetic data on absorption, distribution, metabolism and thus the amount of a chemical or metabolite that is available to the target organ. A lower uncertainty factor would then be required to account for differences between species in sensitivity to a chemical. For reference concentration (RfC) estimates for noncancer endpoints, EPA has usually applied an uncertainty factor of 3 (Jarabek, 1994; Jarabek, 1995; U.S. Environmental Protection Agency, 1994; U.S. Environmental Protection Agency, 2002).

Prior to the new EPA carcinogen risk assessment guidelines, cancer risk at 10^{-5} often was used in regulatory practice as a safe dose, and this was estimated using a linearized multistage model fit of tumor incidence data (Table 3, I). Under the new EPA guidelines, if data are not sufficient for a nonlinear dose–response, the guidelines recommend linear extrapolation from the LTD_{10} to zero. Thus, a risk of less than 10^{-5} is estimated at a dose of the $LTD_{10}/10{,}000$ (Table 3, IIA). If a nonlinear dose–response can be justified in the low-dose range, the new guidelines suggest setting a reference dose (presumably with a negligible cancer risk) by dividing the LTD_{10} by uncertainty factors. The uncertainty factors account for extrapolation from animals to humans, inter-individual variation in sensitivity, possibly an additional factor for sensitivity of children, risk reduction from an effect level (LTD_{10}) to a no-effect level, and in some cases modifying factors for different routes of exposure or weak databases. Hence, the total uncertainty factor may include four factors of 10, which results in a reference dose that is 10,000-fold below the LTD_{10}, i.e., $LTD_{10}/10{,}000$ (Table 3, IIB1). This dose is mathemati-

cally equivalent to the dose corresponding to an estimated risk of less than 10^{-5} obtained by linear extrapolation and also approximates the risk level of less than 10^{-5} that would be estimated by the linearized multistage model. If the total uncertainty factor of 10,000 is replaced by 3000 for the purpose of reducing compounded conservatism, then the reference dose is the $LTD_{10}/3000$ (Table 3, IIB2). In such a case, application of the new guidelines in the nonlinear case results in a reference dose that is 3.3-fold higher than the estimated risk of 10^{-5} that would be obtained by using the linearized multistage model. If a total uncertainty factor of less than 3000 can be justified, the reference dose would be more than 3.3-fold higher than the safe dose at 10^{-5}.

For example, if the LTD_{10} is 6 mg/kg/day, then under the new guidelines the estimated safe dose for a linear extrapolation to 10^{-5} is 0.0006 mg/kg/day (6/10,000); if the dose response is nonlinear and the uncertainty factor is 10,000, then the safe dose is still 0.0006 (6/10,000). If the uncertainty factor of 3000 is used then the safe dose is 0.002 (6/3000), i.e., 3.3 times less stringent.

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Appendix A

Cases are considered for specific tumor types, species, sex, and chemical where there are positive trends for tumor incidence versus dose that provide both a lower and upper confidence limit (LoConf, UpConf) for the TD_{50} in the CPDB. Examination of the CPDB shows that the TD_{50} tends to be near the geometric mean of the LoConf and the UpConf. This is a property of the log-normal distribution. Hence, estimates of the TD_{50} may be approximately log-normally distributed.

Approximate confidence limits for a log-normally distributed estimate of the TD_{50} are of the form

$$\log TD_{50} \pm z \cdot s, \tag{A.1}$$

where s is the standard deviation for $\log(TD_{50})$ and z is a standard normal deviate corresponding to a specified percentile of the distribution. The CPDB lists two-sided 99% confidence limits for the TD_{50}, i.e., 99.5% one-sided limits. For that percentile, $z = 2.575$. From Eq. (A.1), an estimate of s is given by

$$s = [\log(TD_{50}) - \log(LoConf)]/2.575. \tag{A.2}$$

For 95% one-sided confidence limits generally used for benchmark doses, $z = 1.645$. Thus, the approximate lower 95% confidence limit for the TD_{50} is

$$\begin{aligned} LTD_{50} &= \text{antilog}[\log TD_{50} - 1.645\{\log(TD_{50}) \\ &\quad - \log(LoConf)\}/2.575] \\ &= (TD_{50})^{0.36} \times (LoConf)^{0.64}. \end{aligned} \tag{A.3}$$

The next step is to estimate the lower 95% confidence limit for the TD_{10}, i.e., the LTD_{10} that may be used as the point of departure for low-dose cancer risk assessment (U.S. Environmental Protection Agency, 1996). Estimates of the TD_{50} in the CPDB are based on fitting the one-hit model to tumor incidence data

$$P = 1 - (1 - P_0)e^{-qd}, \tag{A.4}$$

where P is the probability of a tumor by a specified age, generally two years for chronic animal bioassays, with a daily exposure to a dose of d units of a chemical, P_0 is the background tumor incidence, and q is the cancer slope factor estimated from bioassay data. Cancer risk in the CPDB is expressed as extra risk $(P - P_0)/(1 - P_0)$. From Eq. (A.4)

$$(P - P_0)/(1 - P_0) = 1 - e^{-qd}. \tag{A.5}$$

The upper 95% confidence limit for q, denoted by q^*, provides the lower 95% confidence limit for the dose at a specified risk. For an extra risk of 50%,

$$0.5 = 1 - e^{-q^* LTD50}, \quad q^* = -\log_e 0.5/LTD_{50}. \tag{A.6}$$

For an extra risk of 10%,

$$0.1 = 1 - e^{-q^* LTD10}, \quad LTD_{10} = -\log_e 0.9/q^*. \tag{A.7}$$

Substituting the value for q^* from Eq. (A.6) gives

$$\begin{aligned} LTD_{10} &= [\log_e 0.9/\log_e 0.5] \times LTD_{50} \\ &= LTD_{50}/6.6. \end{aligned} \tag{A.8}$$

Substituting from Eq. (A.3) for the LTD_{50} gives an approximate value for the LTD_{10} in terms of results listed in the CPDB

$$LTD_{10} = (TD_{50})^{0.36} \times (LoConf)^{0.64}/6.6. \tag{A.9}$$

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