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Calculation of 2,3,7,8-TCDD Equivalent Concentrations of Complex Environmental Contaminant Mixtures

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Sufficient toxicological data are now available to permit use of conventional risk assessment techniques to estimate the hazards associated with human exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). However, many real-world exposures involve complex mixtures of dibenzodioxins, dibenzofurans, and related compounds. Historical approaches to risk assessment on such mixtures have ranged from ignoring all compounds except 2,3,7,8-TCDD itself to assuming that all compounds have potencies equal to 2,3,7,8-TCDD. An alternative approach which uses existing literature data and analytical results to calculate the "2,3,7,8-TCDD equivalent" concentration of a mixture in order to "predict" its biological potency relative to 2,3,7,8-TCDD itself is advanced here. Previously reported *in vivo* acute and subchronic studies and some recently obtained analytical chemistry data are integrated here to clarify the utility of this important approach and to assess the uncertainties associated with its use. This predictive approach, and various conceptually similar ones, have now found wide applicability to the risk assessment process associated with exposure to complex mixtures of dioxins, dibenzofurans, and related compounds.

Introduction

Sufficient toxicological data are now available to permit use of conventional risk assessment techniques to estimate the hazards associated with human exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) (*1*). In a few incidents, 2,3,7,8-TCDD has been the only congener present at toxicologically significant concentrations. More commonly, however, real-world exposures involve complex mixtures of compounds with dioxin-like activity containing up to 75 different chlorinated dibenzo-*p*-dioxin (PCDD), 135 chlorinated dibenzofuran (PCDF), and 75 chlorinated biphenylene (PCBE) isomers and congeners. For example, commercial polychlorinated biphenyls (PCBs) (*2*), emissions from garbage-burning resource recovery plants (*3*), and chemical waste incinerators (*4*), foodstuffs (*5*), human milk (*6*), and pyrolyzed PCBs or chlorobenzenes (*7,8*) all consist of complex mixtures in which 2,3,7,8-TCDD is a minor component relative to the total PCDD, PCDF, and PCBE concentration.

Such mixtures present a difficult problem for estimating human health hazards for at least three reasons. First, analytical chemistry has not yet progressed to the point that reliable isomer-specific quantitation or even qualitative identification is possible for the full range of dioxin-like compounds. For example, the unlabeled standards needed both for qualitative identification and for determination of response factors are not generally available. In addition, only a very limited set of ^{13}C- or ^{37}Cl-labeled internal standards, which are necessary to correct for run-to-run variations in recovery, have been prepared. Even modern high resolution capillary gas chromatographic techniques do not permit resolution of all possible congeners. Second, insufficient toxicological data are available to permit a rigorous risk assessment for most congeners. For example, two-year carcinogenesis data are available only for 2,3,7,8-TCDD itself, for 2,7-dichlorodibenzodioxin, and for a mixture of two 2,3,7,8-substituted hexachlorodibenzodioxins (hexaCDDs) (*9*). Single oral dose guinea pig LD_{50} values have been reported for 16 different dioxins and five different furans (*10*). Fewer than 25 of the 75 possible dioxin and 20 of the 135 possible furan congeners have been subjected to *in vitro* determination of biological activity (*10*). Finally, there exists a third confounding factor. The biological potency of the mixture may not

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simply equal the summation of the activities of the individual congeners present, if synergistic of antagonistic interactions among the mixture's components are significant.

On February 5, 1981, a fire involving a transformer containing 60% Aroclor 1254 and 40% tri- and tetrachlorobenzenes extensively dispersed a fine oily soot throughout a 17 story office building in Binghamton, New York. Chemical analysis demonstrated the presence of substantial concentrations of PCDDs, PCDFs and PCBPs in the soot and on various surfaces (*11–13*). The necessity of establishing reoccupancy criteria for the building focused attention on the need to estimate the biological potency of this complex mixture. Two conceptually opposed methods can be discerned in pre-1981 governmental approaches to this problem. The commonly used early approach (probably encouraged by lack of analytical chemistry data) was explicitly or implicitly to ignore all compounds other than 2,3,7,8-TCDD itself. Alternatively, all compounds detected were assumed equal in potency to 2,3,7,8-TCDD (*14*). Either approach ignores substantial biological evidence suggesting that the potencies of particular congeners vary widely, but are, in some cases, negligible when compared to 2,3,7,8-TCDD. Any realistic attempt to estimate a mixture's biological potency must therefore take into account the relative potencies of its individual components.

The approach eventually developed to estimate the hazard associated with exposure to the Binghamton mixture is a fairly general one. Published short-term biological data are used to estimate the ratio of the potency of a particular congener relative to 2,3,7,8-TCDD. Then, the observed or estimated concentration of that congener is multiplied by this ratio to estimate the "2,3,7,8-TCDD equivalent" concentration of that congener. This 2,3,7,8-TCDD-equivalent concentration of a particular congener would thus be equated with a hypothetical concentration of 2,3,7,8-TCDD capable of producing the same biological effect as the actual concentration of that congener. The overall biological potency of the mixture could then be estimated by summing the TCDD-equivalent concentrations of all congeners present. Such an approach is only applicable when the components of the mixture exhibit biological effects similar to those of 2,3,7,8-TCDD.

When first advanced (*15*), the TCDD-equivalent approach was novel; more recently, conceptually similar approaches have been used in assessing other PCB-related incidents, e.g., the One Market Plaza incident in San Francisco (*16*), and in assessing other sources of environmental exposure, e.g., emissions from resource recovery plants (*17*), and these have gained increasing acceptance as general solutions to the PCDD/PCDF mixture problem (*18*). However, it must be emphasized that the only direct experimental support for this approach rests on work performed in connection with the Binghamton incident. Here alone was sufficient contaminated material (i.e., "soot") and appropriate laboratory resources available to allow *in vivo* comparisons of an environmental mixture's biological potency relative to 2,3,7,8-TCDD itself, and thus verification of the TCDD-equivalents calculation. The intent of this paper is to present an integrated picture of the previously reported biological experiments (Table 1) and some recently obtained analytical chemistry data (Table 2) to clarify the utility of this important approach.

Table 1. Influence of structure and chlorination pattern on guinea pig oral LD_{50} (male, Hartley, 200–250 g).

Compound	LD_{50}, μg/kg	Ratio LD_{50} compound / LD_{50} 2,3,7,8-TCDD
2,3,7,8-TetraCDD	2.5[a]	1
2,3,7,8-TetraCDF	5–10[b]	2–4
2,3,4,7,8-PentaCDF	<10[c]	<4
1,2,3,7,8-PentaCDD	3.1[c]	1.2
1,2,3,4,7,8-HexaCDD	73[c]	29
1,2,3,7,8,9-HexaCDD	60–100[c]	24–40
1,2,3,7,8-HexaCDD	70–100[c]	28–40
2,3,4,6,7,8-HexaCDF	120[c]	48
1,2,3,4,6,7,8-HeptaCDD	<600[c]	<240
1,2,4,7,8-PentaCDD	1,125[c]	450
2,3,7-TriCDD	29,444[c]	1,200
2,8-DiCDD	730,000[c]	300,000
1,3,6,8-TetraCDD	>15,000,000[d]	600,000

[a] Data of Silkworth et al. (*19*).
[b] Data of Moore et al. (*20*).
[c] Data of McConnell et al. (*21*).
[d] Data of Kawamura et al. (*22*).
[e] Data of McKinney et al. (*23*).

Calculation of TCDD Equivalents from Chemical Data

Chemical data can be used to estimate the 2,3,7,8-TCDD equivalent concentration present in a mixture, provided that the concentrations of the relevant compounds are known and that their biological activities can be estimated. A biological end point of special concern for these compounds is carcinogenesis (*1*); unfortunately, only five dibenzodioxins and no dibenzofurans have been the subject of lifetime animal bioassays (*10*). Thus, short-term bioassay data, which are substantially more numerous, must be utilized as the basis of the equivalence calculation. The end point used in the present study to estimate relative congener potency is the single-dose oral LD_{50} in the guinea pig. This is advantageous, since the LD_{50} of the mixture in question (soot) has been experimentally determined (*24*), thus allowing direct verification of the calculation. However, since there is good correlation among various short-term assays for these compounds (*25–29*) any differences between results from particular short-term assays should have only minor effects on the calculation (*18*).

The issue of the relevance of short-term exposure data to carcinogenic potency is less clear. However, the very limited experimental data now available supports the concept that short- and long-term effects are roughly proportional. Thus, a mixture of two 2,3,7,8-substituted hexaCDDs exhibited carcinogenic potency

Table 2. Concentrations of chlorinated dibenzo-*p*-dioxins and dibenzofurans in the Binghamton soot sample used in animal toxicology experiments.[a]

Compound	Concentration, ppm
Total diCDD	0.5
Total triCDD	0.5
Total tetraCDD	1.5
2,3,7,8-tetraCDD	0.5
Total PeCDD	1.4
1,2,3,7,8-PeCDD[b]	0.5
Total HxCDD	1.2
1,2,3,4,7,8-HxCDD	0.2
1,2,3,6,7,8-HxCDD	0.2
1,2,3,7,8,9-HxCDD	0.2
Total HpCDD	1.4
1,2,3,4,6,7,8-HpCDD	0.7
Total OCDD	1
Total diCDF	5.9
Total triCDF	27
Total tetraCDF	120
2,3,7,8-tetraCDF	17
Total PeCDF	160
1,2,3,7,8-PeCDF	22
2,3,4,7,8-PeCDF	12
Total HxCDF	64
1,2,3,4,7,8-HxCDF	26
2,3,4,6,7,8-HxCDF	1.2
1,2,3,6,7,8-HxCDF	7.7
1,2,3,7,8,9-HxCDF	1.5
Total HpCDF	26
1,2,3,4,6,7,8-HxCDF	10
Total OCDF	13

[a] Unless otherwise indicated, concentrations listed are based on a recent redetermination of the PCDD/PCDF constituents in the soot using contemporary analytical methodology. This redetermination is considered more reliable than that previously reported (*32*), particularly in view of recent advances in the availability of labeled and unlabeled standards and chromatography.
[b] Estimated from data of Silkworth et al. (*32*).

estimated to be 0.04 times that of 2,3,7,8-TCDD (*18*); the ratio of corresponding guinea pig acute oral LD_{50} values is 0.03. Similarly, in two-year studies of unsubstituted dibenzodioxin (*30*) and 2,7-dichlorodibenzodioxin (*31*), doses up to 10,000 ppm in the diet elicited no carcinogenic response in either species. Unchlorinated and dichlorinated dibenzodioxins are essentially inactive relative to 2,3,7,8-TCDD in a battery of short-term bioassays (*10*).

Table 1 presents the currently available data on guinea pig acute oral LD_{50} values for dibenzodioxins and dibenzofurans. Use of this data set to predict the 2,3,7,8-TCDD equivalent concentration in a mixture (such as the Binghamton soot) is complicated by certain limitations, particularly the absence of any data on PCDFs other than 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 2,3,4,6,7,8-HxCDF. The following assumptions will therefore be made about LD_{50} values not yet determined.

(1) The ratio of the LD_{50} values of a particular PCDF congener and 2,3,7,8-TCDF will be the same as the ratio of the LD_{50} values of the correspondingly substituted PCDD congener and 2,3,7,8-TCDD. There is no direct experimental data to support this assumption.

(2) The LD_{50} values of PCDFs and PCDDs lacking chlorines at any of the four lateral positions will be sufficiently high that their influence can be ignored for most environmental mixtures. This assumption is based on the guinea pig LD_{50} values of 2,8-diCDD, 2,3,7-triCDD, and 1,3,6,8-tetraCDD and 1,2,4,7,8-pentaCDD. All have LD_{50} values more than 450 times higher than 2,3,7,8-TCDD itself (Table 1). This simplification may be invalid for mixtures containing only very small percentages of 2,3,7,8-substituted isomers.

(3) Introduction of a single additional chlorine substituent on a 2,3,7,8-substituted congener has essentially no effect on the congener's guinea pig LD_{50}. This assumption is based on comparison of the LD_{50} values of 2,3,7,8-TCDD versus 1,2,3,7,8-pentaCDD, and 2,3,7,8-TCDF versus 2,3,4,7,8-pentaCDF (Table 1).

(4) Introduction of two additional chlorine substituents on a 2,3,7,8-chlorinated congener raises its LD_{50} by a factor of 30. This assumption is based on comparison of the LD_{50} values of 1,2,3,4,7,8-, 1,2,3,6,7,8-, and 1,2,3,7,8,9-hexaCDD versus 2,3,7,8-TCDD (Table 1).

(5) The LD_{50} values of compounds with more than six chlorines will be sufficiently high that their influence can be ignored for most environmental mixtures. This assumption is based on comparison of the LD_{50} values of 1,2,3,4,6,7,8-heptaCDD and 2,3,7,8-TCDD (Table 1).

These assumptions require that attention be focussed only on 2,3,7,8-substituted PCDDs and PCDFs. The concentration of 2,3,7,8-TCDF in a bulk soot sample used in the animal toxicology experiments described later has recently been measured at 17.1 ppm (Table 2); since the data in Table 1 indicate that the LD_{50} value of 2,3,7,8-TCDF is about three times that of 2,3,7,8-TCDD, this is equivalent in terms of acute toxicity to a 2,3,7,8-TCDD concentration of about 6 ppm. Based on assumption (2), other tetraCDFs can be neglected in this calculation. The two pentaCDF isomers with 2,3,7,8 substitution together total 34 ppm. If, as is required by assumption (3) and as is consistent with experimental data for 2,3,4,7,8-pentaCDF, the toxicity of 2,3,7,8-substituted pentaCDFs is considered equal to that of 2,3,7,8-TCDF, this concentration corresponds to a 2,3,7,8-TCDD equivalent concentration of about 11 μg/g. The hexaCDFs were measured at 64 μg/g. However, based on assumption (2), only 2,3,7,8-substituted congeners (1,2,3,4,7,8-, 1,2,3,6,7,8-, and 2,3,4,6,7,8-hexaCDF) need be considered. Table 1 suggests that 2,3,4,6,7,8-hexaCDF is about 16 times less potent than 2,3,7,8-TCDF itself. Thus, the sum of observed 2,3,7,8-hexaCDF concentrations (36 μg/g) corresponds to a 2,3,7,8-TCDD equivalent concentration of about 1 ppm (Table 3). Hepta and octaCDFs have insufficient concentrations and biological potencies to contribute to this calculation. The concentrations of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD have each been measured at 0.5 μg/g, corresponding to a "2,3,7,8-TCDD" equivalent concentration of 1 ppm. The concentration of hexaCDDs (1.2 μg/g), when adjusted for biological potency by dividing by 30, makes a negligible contribution to the calculation. Similarly, hepta and octaCDDs can be ignored.

Table 3. Calculation of 2,3,7,8-TCDD equivalents due to various chlorinated dibenzofurans, dibenzodioxins, and biphenylenes in Binghamton soot using chemical data.[a]

C Concentration	*A* Relative activity compound class vs. *dibenzodioxins*	*B* Relative activity due to chlorine *substitution*	2,3,7,8-TCDD equivalents, *ppm*
2,3,7,8-TCDF/ 17.1 ppm	1/3	1	6
1,2,3,7,8- and 2,3,4,7,8-PeCDF/33.8 ppm	1/3	1	11
1,2,3,4,7,8-, 2,3,4,6,7,8-, 1,2,3,6,7,8-, and 1,2,3,7,8,9-hexaCDFs/36 ppm	1/3	1/16	1
2,3,7,8-TCDD/0.5 ppm	1	1	0.5
1,2,3,7,8-PeCDD/ 0.5 ppm	1	1	0.5
2,3,6,7-TCPB/1.1 ppm	1	1	1
1,2,3,6,7-PeCPB/ 2.3 ppm	1	1	2

[a] 2,3,7,8-TCDD calculated as the product $C \times A \times B$.

Together, the dibenzodioxins and dibenzofurans account for about 19 μg/g "2,3,7,8-TCDD equivalents." Stalling's demonstration (*12*) that the soot contains a substantial concentration of PCBEs complicates the calculation substantially. The only data available relevant to the biological potency of these derivatives are measurements of the cytosolic receptor binding affinity and the cell keratinization activity of 2,3,6,7-tetraCBE (analog of 2,3,7,8-TCDD) (*33*). These results suggest that 2,3,6,7-tetraCBE may have a biological potency similar to that of its structural analog, 2,3,7,8-TCDD. Unfortunately, nothing is known about the rate of metabolic detoxification of these compounds. However, in the absence of more rigorous data, it will be assumed that biphenylenes exhibit the same biological potency as the corresponding dibenzodioxins, and three times the potency of the corresponding dibenzofurans.

Even today, quantitative determination of the PCBE concentration in the soot is complicated by the absence of any reliable labeled or unlabeled biphenylene standards. As a crude estimate of their concentration, Stalling's observation that the PCBEs are present at approximately 1/15 the concentration of the PCDFs can be used. This factor, coupled with the foregoing assumption that their biological potency is three times that of the dibenzofurans implies a contribution of about 3.4 ppm from the biphenylenes. Thus, the best estimate of the overall potency of the mixture of PCDDs, PCDFs and PCBEs is ca. 22 ppm (Table 3).

Calculation of 2,3,7,8-TCDD Equivalents from Biological Data

The best test of the various assumptions underlying the calculation of TCDD equivalents from chemical data is to perform an empirical determination of the mixture's biological potency versus 2,3,7,8-TCDD itself. Since the estimate of biological potency was based on acute guinea pig oral LD_{50} data, the most direct point of comparison requires experimental measurement of this parameter for the mixture. Female guinea pigs were administered a single oral dose of a benzene extract of the soot equivalent to 4, 20, 100, 500, or 1000 mg of soot/kg (*24*). The soot extract's LD_{50} was determined to be 327 mg/kg, based on a 42-day observation period and calculated by a modification of the method of Bliss (*34*). For purposes of comparison, female guinea pigs were similarly given single oral doses of 0.1, 0.5, 2.5, 12.5, or 20 μg/kg 2,3,7,8-TCDD (*24*). The corresponding calculated LD_{50} value for 2,3,7,8-TCDD was 19 μg/kg in an aqueous vehicle. Thus, by using the relationship (1),

$$\text{2,3,7,8-TCDD-equivalent concentration } (\mu g/g) = \frac{LD_{50} \text{ for 2,3,7,8-TCDD } (\mu g/g)}{LD_{50} \text{ for soot (mg/kg)}} \quad (1)$$

it can be calculated that the soot has a 2,3,7,8-TCDD equivalent concentration of (19 μg/kg)/(327 × 10^3) μg or 58 ppm.

A more demanding test of the TCDD-equivalent concept is to calculate the TCDD equivalent concentration of the soot based on a variety of exposure end points induced subchronically. Thus, Binghamton soot was incorporated into feed at concentrations of 0, 0.2, 1.9, 9.3, 46.3, and 231.5 ppm and fed to guinea pigs for 90 days (*34*). In another study, 2,3,7,8-TCDD was incorporated into feed at concentrations of 0, 2, 10, 76, and 431 ppt and similarly fed to guinea pigs for purposes of comparison (*35*). End points chosen were those in which significant, dose-related differences from control values were observed after subchronic exposure in both studies. These endpoints included relative (to body) thymus weights (males), percent of initial body weight (males), triglyceride levels (males) serum ALT levels (females), and incidence of hepatocellular cytoplasmic inclusion bodies (females). For the continuous data parameters, linear regression analysis was performed to obtain the best straight-line fit for exposure (expressed as ppt 2,3,7,8-TCDD or log ppt 2,3,7,8-TCDD in the feed) versus response using data from the 2, 10, and 76 ppt dose groups in the present study. Correlation coefficients were obtained for each line. Response data were normalized by expressing each value as a percentage of the corresponding control value. This was necessary to correct for small variations between the control values in each study.

Response data for a particular dose level in the Binghamton soot study were then compared to the standard curves generated above to obtain a hypothetical 2,3,7,8-TCDD concentration in the feed which would have been expected to produce the same degree of response. The "2,3,7,8-TCDD equivalent" concentration of the soot was then calculated by using Equation (2). The particular dose levels of Binghamton soot chosen for this comparison were those in which the degree of response fell

within the limits of the corresponding standard curve (1.9 ppm soot level was used).

$$\text{2,3,7,8-TCDD-equivalent concentration } (\mu g/g) = \frac{\text{hypothetical 2,3,7,8-TCDD concentration in feed (pg/g)}}{\text{concentration of Binghamton soot in feed } (\mu g/g)} \quad (2)$$

A different method was employed for analysis of the quantal data obtained for the incidence of hepatocellular cytoplasmic inclusion bodies. In this case, use was made of the ED_{50} (dose level in feed expected to produce a 50% incidence) in order to facilitate comparison of pure 2,3,7,8-TCDD with the Binghamton soot. The ED_{50} following 90-day exposure to the soot was calculated using previously reported incidence data (*39*) by a modification of the method of Bliss (*36*). A corresponding ED_{50} for subchronic 2,3,7,8-TCDD exposure could not be calculated from results of the present study since a range of incidences was not obtained. For purposes of comparison, 76 ppt was assumed to be the ED_{50} dose level for 2,3,7,8-TCDD, since 50% of female animals at this dose level exhibited inclusions at a severity grade of +1 or greater as previously defined (*34,35*). The 2,3,7,8-TCDD equivalent concentration (ppm) of the soot was then calculated as:

$$\text{2,3,7,8-TCDD-equivalent concentration } (\mu g/g) = \frac{ED_{50} \text{ for 2,3,7,8-TCDD (ppt)}}{ED_{50} \text{ for soot (ppm)}} \quad (3)$$

Finally, an effective LD_{50} value for prolonged 2,3,7,8-TCDD exposure was similarly derived by using total dose versus mortality data from a recovery experiment in the previous investigation (*34*). This was compared with corresponding calculations from the soot study to yield an equivalent concentration (ppm) by using Equation 1.

Table 4 summarizes the results of calculations of the 2,3,7,8-TCDD equivalent concentration of Binghamton soot based on dose-response data from previous investigations (*34,35*). Excellent dose-response correlations ($r > 0.950$) were obtained for the continuous data endpoints (relative thymus weight, % of initial body weight, serum triglycerides, and serum ALT) after 2,3,7,8-TCDD exposure. Log transformation of the 2,3,7,8-TCDD feed concentrations was required for a good fit only in the case of serum ALT levels. Calculated "2,3,7,8-TCDD-equivalent" concentrations for the Binghamton soot were in good relative agreement for three of the four end points, with values ranging from 18 to 21 ppm (μg 2,3,7,8-TCDD/g soot). Calculations based on serum triglyceride levels resulted in a somewhat lower value of 5 ppm.

Data on the incidence of hepatocellular cytoplasmic inclusion bodies in female guinea pigs fed Binghamton soot were employed to determine a value of 7.6 ppm (2.7 to 21.6 ppm, 95% confidence range) as the ED_{50} dose level of soot for this lesion. Using a value of 76 ppt for the ED_{50} dose level following exposure to pure 2,3,7,8-TCDD, the "2,3,7,8-TCDD-equivalent" concentration of the soot was determined to be 10 ppm (4 to 28 ppm, 95% confidence limits). A similar approach was employed for calculation of an effective LD_{50} for soot exposure, except that total soot consumption was used instead of soot concentration in the feed. This LD_{50} dose was determined to be 383 mg soot/kg (186 to 790 mg/kg, 95% confidence range). Comparison with the effective LD_{50} of 0.8 μg 2,3,7,8-TCDD/kg (0.6 to 1.1 μg/kg, 95% confidence limits) yielded a value of 2 ppm (1 to 3 ppm, 95% confidence limits) for the 2,3,7,8-TCDD-equivalent concentration of the Binghamton soot.

Table 4. Calculated 2,3,7,8-TCDD-equivalent concentrations of the Binghamton soot for various dose-related endpoints following subchronic exposure.[a]

End point	Sex	Method[b]	r[c]	2,3,7,8-TCDD equivalent concentration in soot, ppm
Relative thymus weight (decrease)	M	Linear	0.951	19[d]
% of initial body weight (decrease)	M	Linear	0.994	21[d]
Serum triglycerides (increase)	M	Linear	0.998	5[d]
Serum ALT (decrease)	F	Log	0.960	18[e]
Hepatocellular cytoplasmic inclusion bodies	F	ED_{50}	—	10 (4–28)[f]
Mortality	M	LD_{50}	—	2 (1–3)[f]

[a] Response data from the previously reported Binghamton soot (*34*), and 2,3,7,8-TCDD (*35*) subchronic studies 2,3,7,8-TCDD-equivalent concentrations for the soot.

[b] Method by which 2,3,7,8-TCDD dose-response standard curve was constructed: Linear = linear regression analysis of dose level (ppt) vs. response; LOG = linear regression analysis of log dose level (ppt) vs. response; ED_{50} = calculation of ED_{50} dose level by method of Carmines et al. (*36*); LD_{50} = calculation of LD_{50} for subchronic exposure by method of Carmines et al. (*36*).

[c] Correlation coefficient from linear regression analysis.

[d] Calculated using response data from 1.9 ppm Binghamton soot dose level.

[e] Calculated using response data from 3.9 ppm Binghamton soot dose level.

[f] 95% confidence limits.

Comparison of Chemical and Biological Calculations

The most direct test of the assumptions underlying the calculation of 2,3,7,8-TCDD equivalents from chemical data and published acute oral LD_{50} values is comparison with the biological calculation based on the acute oral LD_{50} of the mixture and of 2,3,7,8-TCDD itself. The chemical data-based 2,3,7,8-TCDD equivalents estimate of 22 ppm is in reasonable agreement with the experimentally determined value of 58 ppm.

From a practical perspective, however, the predictions of magnitude of acute toxicity expressed by a mixture is of less interest than prediction of the mixture's subchronic and chronic toxicity. The six subchronic endpoints listed in Table 4, although not necessarily representing adverse effects of great clinical importance, nevertheless serve as sensitive indicators of exposure.

The calculated equivalent concentration values varied over an approximately tenfold range (2 to 21 ppm), depending upon the end point chosen. Such a range is not unexpected, since various soot components might be more or less effective than 2,3,7,8-TCDD in producing a specific end point. While the soot appeared to be very effective in causing alterations in thymus weight, body weight, and serum triglyceride levels, it was less effective at causing death. Taken together, these findings suggest that biologically based calculations of 2,3,7,8-TCDD equivalent concentrations are best based on a range of biological end points.

It is notable that for the Binghamton soot, use of the acute oral LD_{50} as the end point resulted in higher apparent toxicity (i.e., the highest 2,3,7,8-TCDD equivalent concentration) than the various subchronic endpoints. Thus, use of the acute LD_{50} or use of the chemical data to perform a calculation may provide a conservative estimate of the acute or subchronic activity of the mixture. However, the observation that the 2,3,7,8-TCDD equivalent concentration varied by about an order of magnitude when calculated by using various subchronically induced end points is an indication of the uncertainty associated with the use of any single biological end point or of chemical analysis data alone to estimate a mixture's potency.

Conclusions

The acute oral LD_{50} of a soot sample containing a complex mixture of PCDDs, PCDFs, and PCBEs has been shown to correspond to that of a hypothetical material containing only 2,3,7,8-TCDD at 58 ppm. The corresponding 2,3,7,8-TCDD equivalent concentration calculated by using various dose-related subchronic end points ranged from 2 to 19 ppm. Conversely, using existing literature data to estimate the potency of individual congeners and the results of chemical analyses on the soot, it can be "predicted" that the soot contains ca. 22 ppm 2,3,7,8-TCDD equivalents. In light of uncertainties in the chemical analyses and toxicological determinations, as well as uncertainties in the sample congener toxicity data upon which this calculation relies, the agreement between observed and predicted concentrations is good. However, the substantial variation in the results of the "equivalents" calculation depending on endpoint chosen clearly shows that any determination based on a single end point or any calculation based on chemical concentrations has a substantial uncertainty associated with it. Nevertheless, these uncertainties will often be small in comparison to the many uncertainties and approximations inherent in a risk assessment for a single compound, such as 2,3,7,8-TCDD. It appears likely that this approach, or a conceptually similar one, will find increasing use in the evaluation of the hazard posed by complex mixtures.

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