Cancer risk assessment for TCDD and other compounds must focus on the cancer dose-response relationship and corresponding potency for the range of human doses before it can have relevance to the human exposure environment. Major differences of opinion exist over whether the dose-response curve for TCDD and other dioxin congeners is non-linear (incorporating a threshold dose region below which tumors are unlikely to be elicited) or linear (implying that any exposure has a statistical likelihood of causing cancer). The World Health Organization and others strongly support a non-linear dose-response relationship for TCDD and cancer, whereas USEPA characterizes the dose-response function as linear. This review critically summarizes the available information on TCDD dose-response relationship for cancer utilizing a weight-of-evidence approach. This assessment concludes that the available data support a non-linear dose-response relationship as being most likely and appropriate for human cancer risk assessment, i.e., the evidence suggests that a biological threshold exists in the dose-response. While proof of a threshold is not absolute, and never can be, the level of certainty for TCDD is substantial because of the concordance of many lines of evidence and the consistency of repeated observations pointing to non-linearity.

Key Words: cancer; TCDD; dose-response; non-linearity; linearity; cancer threshold.

Major differences of opinion exist over whether, at low doses, the dose-response curve for the carcinogenicity of TCDD and other dioxin congeners is non-linear (incorporating a biological threshold dose range below which tumors are unlikely to be elicited in humans) or linear (implying that any dose is capable of imparting a statistical likelihood of cancer among exposed individuals). The World Health Organization and others (Canady et al., 2001; ECSCF, 2000, 2001; JECFA, 2001; van Leeuwen et al., 2000; WHO, 1991) strongly support a non-linear dose-response relationship for TCDD and cancer, whereas USEPA (2003) characterizes the dose-response function as linear, despite summarizing extensive biological data including those related to the Ah receptor (AhR) that support a non-linear threshold mode of action (MoA).

Risk estimation for TCDD requires information on the nature and magnitude of the carcinogenic potency, as for any other xenobiotic. A useful measure of carcinogenic potency is defined by the shape of the dose-response relationship in the range of doses relevant to human exposures, and these doses are well below those at which tumors have been elicited in laboratory rodents. In this article, we review the pertinent data and judge whether a non-linear or linear approach best fits the available data.

TOXICOLOGICAL BASIS FOR NON-LINEARITY AND THRESHOLD DOSE-RESPONSE RELATIONSHIPS

Several complementary avenues of evidence support a non-linear shape of TCDD’s dose-response relationship for cancer: (1) receptor-mediated responses, (2) modes of carcinogenic action in rodents (genetic versus epigenetic, hepatotoxicity related to carcinogenesis), (3) human evidence, and (4) experimental evidence (in laboratory animals) of non-linearity at low doses.

Receptor-Based Toxicity of TCDD

Toxicologic MoA determines whether a dose-response is linear or non-linear and whether biological thresholds are likely to exist (Brown, 1990; Hoffman and Lefkowitz, 1990; Taylor, 1990). A broad consensus has emerged that TCDD produces cancer in laboratory animals via the interaction with AhR to a degree sufficient to produce specific cellular and tissue toxicity, the repair and regeneration of which lead secondarily to tumor formation (USEPA, 2003; van Leeuwen et al., 2000; WHO, 1991). Tissue concentrations of xenobiotics are monotonic increasing functions of dose rate.

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2 Not all forms of TCDD-induced responses, such as induction of xenobiotic metabolizing enzymes, are the result of the same interactions as those ultimately responsible for cancer, although binding to AhR is commonly involved.
Receptor-ligand interactions are governed by the law of mass action, so a monotonic increasing relationship exists between the tissue concentration of xenobiotic and the number or fraction of receptor-ligand complexes. Since a minimum number or fraction of compound-receptor complexes are needed to elicit an adverse reaction and these effects depend on the fraction of receptors occupied above some threshold for that effect, a threshold in the dose-response relationship is expected for receptor-mediated MoA for TCDD as with all other receptor mediated biological responses. Binding affinity between agonist and receptor and intrinsic activity of the agonist play vital roles in determining the location of thresholds for a given effect (Ariens et al., 1960).

The TCDD/AhR complex and other protein factors enter the nucleus, and alter transcription of specific genes via alteration of mRNA. Genes affected directly and indirectly by the TCDD/AhR-complex code for both inhibitory and stimulatory growth factors and their gene products affect cellular growth and differentiation leading to tumor promotion and carcinogenicity as well as other forms of toxicity (Andersen et al., 1994; Gaido et al., 1992; Poland, 1996; Schwarz and Appel, 2005; Sutter et al., 1991). The identity of specifically altered genes has been reported (Whitlock, 1999), as have numerous additional components of the AhR complex (Carlson and Perdew, 2002; Kumar and Perdew, 1999; Mimura et al., 1999; Petrulis and Perdew, 2002). Molecular modifications arising from binding to the AhR also have been reported as possible steps in the carcinogenicity of TCDD (Carlson and Perdew, 2002; Enan et al., 1998; Matsumura, 2003).

Crump et al. (1976) suggested that, in general, xenobiotic-mediated cancer responses caused by the same mechanism as an underlying background tumor rate must have a linear dose-response at sufficiently low doses. However, if an agent’s MoA for cancer causation for a particular tumor differs from the MoA causing the background incidence, or if there is no background incidence then the general argument does not apply. This situation applies to TCDD and most notably for TCDD-induced hepatocellular adenomas, which are apparently mediated through tissue damage, resulting in substantial cell proliferation at high doses which is not observed at low doses or in the normal rodent liver. The same may also hold for other promoters that produce reversible adducts. Indeed, the high endoxin\(^3\) toxic equivalent quantity (TEQ) (Connor et al., 2004; Schecter and Olson, 1997) provides evidence that the body contains homeostatic defense mechanisms that TCDD and other dioxins must overcome before affecting cellular growth control and causing toxicity including cancer (hence a threshold).

Overall, the evidence indicates that (1) TCDD causes cancer via a receptor-mediated process; (2) this dose-response is nonlinear; and (3) a threshold region exists for TCDD-induced cancer below which adverse effects are unlikely to occur.

**Mode of Carcinogenic Action of TCDD in Rodents**

Laboratory animal findings as they relate to the carcinogenic potential of TCDD include: (1) genotoxicity, (2) tumor promotion, (3) relationship between liver toxicity and carcinogenicity, and (4) low-dose effects.

**Genotoxic evaluation.** TCDD is known to not act via a direct genetic MoA by virtue of the many studies that have overwhelmingly demonstrated a lack of genotoxicity of TCDD. Furthermore, TCDD does not induce DNA adducts as evidenced by at least three studies, one using an ultra-sensitive accelerator mass spectroscopic analysis (Poland and Glover, 1979; Randerath et al., 1988; Turteltaub et al., 1990).

Since the initiation step of carcinogenesis is generally recognized to be secondary to a gene mutation, the results of several studies evaluating TCDD as an initiating agent are relevant to assessing genotoxicity. The negative results from multiple studies assessing initiation of TCDD in both liver and skin strongly limit any hypothesized genotoxicity from either direct or indirect mechanisms (Lucier et al., 1991; Pitot et al., 1980; Poland et al., 1982).

**Tumor promotion evaluation.** In contrast to the extensive negative data on genotoxicity of TCDD, the repeatedly demonstrated tumor-promoting effect of TCDD in the skin and liver strongly supports that the carcinogenicity in rodents results from promotion of independently initiated cells subsequent to tissue damage (Pitot et al., 1985). The tumor promotion MoA of TCDD strongly argues for a threshold for its carcinogenic response (Pitot et al., 1985; Schwarz and Appel, 2005; Whysner et al., 1996).

**Relationship of hepatotoxicity and hepatocarcinogenicity.** Kociba et al. (1978) indicated that the hepatocarcinogenic response of TCDD in rats was related to liver toxicity, specifically in females where the severity of liver toxicity paralleled the incidence of liver tumors. A further independent histopathologic evaluation of female rat livers from this study (Goodman and Sauer, 1992, 251) confirmed this opinion, i.e., “There appeared to be a distinct correlation between the presence of overt hepatotoxicity and the development of hepatocellular neoplasms.” While hepatotoxicity has also been noted in male rats that do not develop liver tumors, Kociba et al. (1978, 294) noted that “these hepatic changes which were more extensive in females than males.” A hepatic tumor response in females and a lack of a hepatic tumor response in males is consistent with this sex difference in magnitude of hepatotoxicity.

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\(^3\) The term “endoxin” refers to naturally-occurring endogenous AhR ligands found in the human diet including some indole carbinols and their derivatives, heterocyclic aromatic amines, flavonoids, carotinoids, vitamin A derivatives, and tryptophan metabolites. Mass and potency estimates for human dietary intake suggest that some effects of endoxins may be greater than those of exodioxins (halogenated aromatic hydrocarbons found in industrial emissions and as residues in food). “Endoxin” was first coined by Safe (1998) and again used by Connor et al. (2004).
Maronpot *et al.* (1993) also identified a dose-response trend for TCDD hepatotoxicity based on an evaluation of replicative DNA synthesis. Furthermore, studies of TCDD-resistant and TCDD-sensitive strains of rats have demonstrated that TCDD hepatotoxicity is related to the development of hepatic enzyme altered foci which have been used as surrogates for developing tumors (Viluksela *et al.*, 2000).

A dose-response relationship clearly exists for TCDD hepatotoxicity, and parallels the dose-response relationship for tumor formation (or formation of foci of cellular alteration as a surrogate of tumor formation). However, the dose-response relationship for other TCDD-induced responses such as enhanced gene expression are different from the dose-response for tumor formation (Maronpot *et al.*, 1993; Viluksela *et al.*, 2000; see Dragan and Schrenk, 2000, for review).

A time-response difference exists between AhR-related enzyme induction and increased cell proliferation (the latter perhaps indicative of cytolethality of hepatocytes) (Walker *et al.*, 1998). As a result of interaction with the Ah receptor, CYP1A1 induction occurs very rapidly after TCDD administration, while increased cell proliferation is not observed for many weeks, suggesting that the cell proliferation is not immediately receptor-mediated but is mediated by a different set of events probably resulting from chronic injury and cell death. The mechanism of the hepatotoxicity has not been fully elucidated although several possibilities exist including compound-mediated oxidative stress. However, the origin of the hepatotoxic response does not alter the importance of the subsequent proliferative response in tumor formation through a tumor promotion action. The observations of disparity of acute events mediated by the receptor and the delayed hepatotoxic response stresses the importance of hepatotoxicity as a requisite step in the development of tumors in contrast to AhR-related enzyme induction.

These results stress the need for greater attention to hepatotoxicity in the pathogenesis of the carcinogenic response of TCDD in contrast to the pathway of AhR-mediated enzyme induction. Using gene expression responses does not accurately represent the risk of developing a tumor.

Since the relationship of hepatocarcinogenicity and hepatotoxicity is strongly supported by multiple lines of evidence as outlined above, hepatotoxicity as a part of MoA should be included in estimating TCDD’s cancer risk for humans.

**Low-dose effects of TCDD in animals.** The dose-response curve at low doses in animals is pivotal to estimating human risk at substantially lower human doses. Numerous studies with TCDD suggest that low experimental doses in animals may actually suppress adverse biological effects occurring at higher doses. While the suppression of toxic effects are frequently minimal (and often statistically not significant), their repeated observations requires serious consideration.

For example, whereas an increase of foci of cellular alteration in the liver is noted with high doses of TCDD following DEN initiation, low doses of TCDD (below 10 ng/kg-day) following such initiation suppress the number of these foci in female Sprague-Dawley rats (Pitot *et al.*, 1987). The volume of liver composed of foci of cellular alteration is also reduced at the low dose of TCDD compared to both higher TCDD doses and controls in DEN-initiated animals. The decreased number and volume of altered hepatic foci under such conditions is not unique to TCDD; the same phenomenon was also noted for cancer promotion by pheno-barbital (Pitot *et al.*, 1987; Whysner *et al.*, 1996). Yet, whether this decrease in foci number and volume results in a decrease in tumor incidence compared to controls is unclear, and would be difficult to test because of the low incidence of spontaneous liver tumors in the Sprague Dawley rat. However, since foci of cellular alteration represent preneoplastic lesions, then such data can serve as a surrogate for tumor response to estimate risk of human cancer is scientifically reasonable.

Low doses of TCDD also suppress hepatocellular proliferation (Teeguarden *et al.*, 1999). Maronpot *et al.* (1993) also observed the same suppression of cell proliferation at 3.5 ng/kg-day of TCDD versus controls in DEN-initiated animals. However, the depression in cell proliferation in the Teeguarden and Maronpot studies was not associated with a reduction in altered hepatic foci even though such a decrease in altered hepatic foci had been previously reported (Pitot *et al.*, 1987) at lower TCDD doses. While no single comprehensive study has evaluated the relationship of cell proliferation to development of foci of cellular alteration and the ultimate development of neoplasms, the weight of evidence suggests that multiple biological effects related to the carcinogenic response that are enhanced at high doses are actually suppressed by low doses of TCDD.

At low doses of TCDD, the reduction of effects (foci of cellular alteration, cell proliferation) that are increased by high doses strongly argues that the dose-responses for TCDD effects are not linear through low doses and supports the concept of a threshold for hepatocarcinogenicity.

**Human Evidence**

The overall picture of human epidemiology results is mixed. The pattern of results may be driven largely by a variety of uncontrolled confounding factors, such as smoking and occupational exposure to other chemicals (Cole *et al.*, 2003). Normally, one would expect the greatest effect in populations that received the highest exposures. However, the study by Bodner *et al.* (2003) of chemical workers found that the standardized mortality ratio for those exposed to sufficient dioxin to produce chloracne (characteristic of high exposures to TCDD) was 0.5 for all cancers and 0.3 for lung cancer. In contrast, some studies of occupationally exposed populations report small elevations in mortality from all cancers, but without notable or consistent elevations for specific cancer sites.

The elevation in mortality for all types of cancers has been interpreted (USEPA, 2003, Part II, Chapter 7, Part A, p. 36) to
represent a late-stage action of TCDD on a wide range of tumors. This suggestion has no precedent, and lacks empirical foundation. Virtually all late-stage or promoting carcinogens (e.g., hepatitis-C virus, asbestos, and estrogens) cause a limited number of forms of cancer (Cole et al., 2003). Further, the animal data are inconsistent with the assumption that TCDD can promote any tumor anywhere in the body, since specific tumor responses are observed in the bioassays and since a generalized increase in the common tumor types, which would be expected if TCDD were a pluripotent promoter, is not seen. If TCDD were acting as a late-stage promoter in humans, our understanding of the dose-response behavior of promoters would lead preferentially to presumption of a threshold and the use of a non-linear model for risk estimation. A detailed exposition by Starr (2001) provides further evidence of how application of a linear model to TCDD does not fit the data, further supporting a non-linear relationship.

TCDD has been designated as a “human carcinogen” (IARC, 1997, 343) based solely on mechanistic considerations focused on the Ah receptor rather than on human epidemiology findings. This approach has been extended, again without precedent, by asserting that TCDD acts as a pluripotential carcinogen by modestly increasing human risk for all cancer while not substantially increasing the risk for any single cancer. While TCDD has been shown to be a multisite carcinogen in several laboratory animals, no indication exists that this is the case in humans.

Overall, no evidence exists from human studies to designate TCDD as a pluripotent carcinogen for humans. The most important studies for the evaluation of the carcinogenicity of TCDD are four cohort studies of herbicide producers (one each in the U.S. and the Netherlands, two in Germany), and one cohort of residents in a contaminated area from Seveso, Italy. These studies involve the highest exposures to TCDD among all epidemiological studies. The multi-country cohort study from IARC is of special interest because it includes three of four high-exposure cohorts and other industrial cohorts (IARC, 1997).

According to IARC, the strongest overall evidence for the carcinogenicity of TCDD is for all cancers combined, rather than for any specific site. The relative risk for all cancers combined in the most highly exposed and longer-latency subcohorts is 1.4. Although IARC indicated that this relative risk does not appear likely to be explained by confounding, relative risks of this magnitude for studies of other substances, particularly with a lower confidence interval at or near one, are generally found to be the result of confounders. Few examples (perhaps only smoking and ionizing radiation in atomic bomb survivors) exist of agents known to cause an increase in cancers at many sites. This lack of precedent for a multi-site carcinogen without particular sites predominating, combined with the very small excess relative risks, means that the epidemiological findings must be treated with caution. On the other hand, the lack of precedent cannot preclude the possibility that in fact TCDD, at high doses, acts as a multi-site carcinogen. However, even if it does, the general population is exposed to levels far lower than those experienced by the industrial populations examined (IARC, 1997).

Cole et al. (2003, 383) reviewed the epidemiological studies cited by IARC (1997), and concluded that the evidence did not support the classification of “limited evidence of carcinogenicity” since the positive associations between TCDD and carcinogenicity were not observed with any degree of consistency. They indicated that the classification of “inadequate evidence of carcinogenicity in humans” was more appropriate based on all evidence (Cole et al., 2003, 383). In an additional twelve epidemiological studies published between 1997 and 2001, the epidemiologic evidence was either poor or contradicted the idea that TCDD is carcinogenic to humans, overall weakening the association between TCDD and human carcinogenesis (Cole et al., 2003).

Further Evidence of Non-Linearity at Low Doses of Dioxin

Statistical analyses were performed on assorted data sets indicative or suggestive of biological changes mediated by TCDD to examine the extent of non-linearity related to carcinogenicity.

Evaluation of human data. Human cancer mortality data are consistent with many dose-response shapes for TCDD, including linear and non-linear models with thresholds as high as 100,000 ppt-years (using the preferred cumulative body-burden metric). The observation of Crump et al. (2003) that estimates of cancer mortality potency in humans increase as higher doses are omitted from a meta-analysis of epidemiologic studies is highly model-dependent; for example, omission of the highest dose group in the Crump meta-analysis leaves results consistent ($p = 0.07$) with zero slope and three distinct background rates, corresponding to a threshold somewhere between the highest and the next highest dose. Furthermore, recent studies indicate a concentration-dependence of TCDD elimination rates in humans, with higher concentrations resulting in higher elimination rates (Aylward et al., 2005). Consequently, the estimates of dose in the occupational cohorts may be substantially understated (Aylward et al., 2004; Emond et al., 2004), and the same effect could lead to considerable misclassification of exposure. The low-dose risk extrapolations based on previous dose estimates for these cohorts probably overestimate risks (because the risk per unit dose is

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4 The $p$-value given here and the significance estimates in Crump et al. (2003) overstate the significance of results, since they are based on the hypothesis that all uncertainties are adequately modeled by Poisson statistics applied to observed numbers of subjects with cancer in the various cohorts. This hypothesis fails to account for uncertainties induced by dose misclassification, or by the differences between cohorts in smoking status, and probably understates the uncertainties substantially.

5 Since no reason exists to expect that the background rates in the three cohorts are identical, no reason exists to set the background rates equal, even if they are not statistically distinct.
overestimated by the low estimates of doses) and substantially underestimate their uncertainty (see footnote 4), even if the dose-response were linear. However, as Starr (2003) has observed neither the original epidemiologic evidence nor the meta-analysis by Crump et al. (2003) support the claim that TEQ exposures close to background levels are likely to be carcinogenic to humans.

**Evaluation of rodent data.** Observations on the measured dose-response curves in different dose ranges for various endpoints (presumably AhR-related only) suggest strongly that dose-response curves for such endpoints (including cancer) should be expected to be highly non-linear. This situation holds particularly in the range of doses tested in cancer bioassays, and in some cases also at much lower doses.

First, even though we consider it inappropriate to combine different types of tumors for mode of action arguments, consider the dose-response curve for all positively affected cancers combined in the NTP (2004) bioassay. This combination of endpoints provides no probative evidence for any particular MoA, although such combinations have been used and may be appropriate for risk assessment purposes; however, the following argument is independent of the biological validity of combination of tumor endpoints for any particular purpose. If individually the major tumors induced by TCDD have linear dose-response curves in the observation range, this combination of tumors must also have a linear dose-response curve. The logical converse of this mathematical fact is that if the combination does not have a linear dose-response curve, at least one of the major tumors induced by TCDD also does not have a linear dose-response curve. In fact, the combined dose-response curve is non-linear and has a threshold in the range of 22 to 28 ng/kg (5 days/week) nominal dose rate, corresponding to an average liver concentration of 4000 to 5000 pg/g (average body burden 275 to 340 pg/g). Thus, at least one major cancer endpoint has to be highly non-linear within the range of test doses.

A second observation supporting non-linearity is of liver weights of the female Sprague Dawley rats in the NTP bioassay (NTP, 2004). The average liver weights of the rats in this bioassay at each age tested have a Michaelis-Menten dose-response curve versus applied dose, with a half-effect nominal dose rate of 3.42 ng/kg (5 days/week), and the liver weights reached a plateau at a dose rate of 10 ng/kg (5 days/week). However, no significant tumor response was seen for doses as high as 22 ng/kg (5 days/week), the first significant response being observed at 46 ng/kg (5 days/week). Thus, at doses substantially below those capable of inducing observable increments in tumor rates, some TCDD-related process appears to be saturated in the liver, although we do not believe this process is related to tumor MoA, resulting in increased liver weights. The processes occurring at high doses in the liver (including carcinogenesis), therefore, might be substantially different from those at low doses, again suggesting non-linearity in dose-response curves.

Close examination of relevant data pertaining to TCDD and carcinogenicity and the application of appropriate statistical analysis provides further evidence that dioxin’s carcinogenesis dose-response structure is non-linear, containing a threshold region.

**USEPA dioxin reassessment’s methodology to establish a cancer slope factor for 2,3,7,8-TCDD.** USEPA’s draft dioxin Reassessment (USEPA, 2003) attempted to establish a cancer slope factor for TCDD using human data and also from laboratory animal data.

For human data, the cancer slope factor for TCDD at low doses was derived by obtaining estimates of ED01 from human cancer mortality in the Hamburg, NIOSH, and BASF cohorts and linearly extrapolating to lower doses. Estimates for ED01 in men are based on three evaluations of epidemiologic studies (Steetenland et al., 2001; Becher et al., 1998; Ott and Zorber 1996) (the first provided two estimates, the second three, and the third one for an ED01 by changing assumptions about dose-response curve shapes). Furthermore, the epidemiologic estimates of relative risk which had been computed for males were then ascribed to females, thereby doubling the number of estimates in the Reassessment.

For animal data, the Reassessment examined two approaches. The first used an earlier analysis (Portier et al., 1984) that used a traditional linear multistage analysis of the Kociba et al. (1978) study in Sprague Dawley rats and the NTP (1982) study in male and female Osborne Mendel rats and male and female B6C3F1 mice. ED01 estimates were given for three tumor types in female and two in male Sprague Dawley rats, one tumor in male and two in female Osborne Mendel rats, and one tumor type in male and four in female B6C3F1 mice.

The second approach used an analysis by Portier and Kohn (1996) relying on the most recent liver pathology evaluations (Goodman and Sauer, 1992; PWG, 1990) of the bioassay by Kociba et al. (1978). Portier and Kohn (1996) reported survival adjusted probabilities of liver tumors. These were evaluated in the context of a simplified 2-stage carcinogenesis model (mutation to an initiated state, growth of initiated cells, and mutation of the initiated cells to a malignant state). In this model it was hypothesized that (1) the initiation

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6 We use the combination here to gain statistical power, while simultaneously losing specificity as to the particular endpoint(s) to which our conclusion applies.
7 Measurements were at weeks 14, 31, and 53, before the time when such weights might be confounded by growth of nodules and neoplasms.
8 This dose rate corresponds to an average liver concentration of approximately 700 pg/g, or an average body burden of 50 pg/g.
9 Data are insufficient in the references cited (Kociba et al., 1978; Sauer and Goodman, 1990) to compute a survival adjustment; such data also do not appear in PWG (1990) or Goodman and Sauer (1992). Our independent evaluation using unpublished data suggests a minor error in one dose group.
rate is directly proportional to CYP1A2 concentration (despite CYP1A2 being unrelated to carcinogenicity or mutagenicity), (2) the birth rate of initiated cells is linearly proportional to activated epidermal growth factor (EGF) receptor concentration, and (3) the mutation rate to a malignant state is a constant. Estimates of CYP1A2 concentration and activated EGF receptor concentration were obtained from a hypothesized mechanistic model (Kohn et al., 1993) applied to the dosing regimen used by Kociba et al. (1978). The five parameters required to link the mechanistic model for concentrations with the two-stage carcinogenesis model were estimated by maximizing the likelihood for the observed survival adjusted tumor responses in the four dose groups (including the control group). The dose-response curve obtained in this way necessarily is linear at low doses and sub-linear at high doses (including the range of doses used in Kociba et al., 1978, 226), and the low-dose slope obtained is determined by the requirement to fit the high-dose observations. As the authors aptly state, “some of the mechanistic assumptions in this model are speculative,” and “[t]he linkage between the PBPK model of Kohn et al. and 2-stage model is also speculative and, undoubtedly, impacts upon the risk projections.”

The shortcomings of the USEPA’s two approaches are evident from the discussions throughout this article. Specifically, estimates based on epidemiological data are apt to be compromised by confounding, by mis-specification of doses, or by other effects. The epidemiological observations are, in any case, entirely consistent with a non-linear dose-response relationship with a threshold considerably greater than normal human doses over the past decade. The “mechanistic” approach to evaluation of rodent data is compromised by hypotheses that (1) disagree with experimental data on mutagenicity, and (2) contradict the MoA proposed even by USEPA; (3) incorrectly suggest that changes in CYP are somehow related to carcinogenesis. Both the “mechanistic” and traditional linear multistage models used to evaluate rodent data are designed to preclude the possibility of a threshold in the experimental range of doses and below. Naturally, no thresholds were found because the models allow for none. Finally, USEPA has yet to include consideration of the latest NTP rat bioassays that provide direct evidence for a non-linear dose-response consistent with a biological threshold.

**DISCUSSION**

Characterization of TCDD and other dioxin congeners’ dose-response characteristics is a vital step in estimating the most likely cancer risk to humans, so the dose-response structure should be defined as objectively and robustly as possible. Policy choices should be clearly identified and not introduced until a stage beyond the completion of the scientific analysis of dose-response.

The USEPA Cancer Guidelines (2005, pp. 3–22) state that: A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses.

The evidence, obtained over decades, related to the cancer potency of TCDD is as compelling as it is comprehensive. Numerous studies have demonstrated that TCDD is not mutagenic. Buttressing the lack of mutagenicity for TCDD are the findings of three investigative teams who, using state-of-the-art methods of analytical detection, demonstrated no formation of adducts between TCDD and DNA. The absence of mutagenicity, coupled with well-substantiated evidence that dioxin-related carcinogenesis results from a series of events characteristic of a classic “promotor,” indicates a MoA expected to exhibit a non-linear dose-response consistent with a biological threshold.

Pathological evidence indicates that the tumor promoting potential of TCDD in rodents is associated with tissue damage, particularly in the liver. The evidence indicates that the dose of TCDD must be of a magnitude that causes substantial liver toxicity to a degree that leads to cell death, repair, regeneration, and then tumors. The association of carcinogenicity with toxicity and cell proliferative repair processes again argues that rodent liver tumor induction has a biological threshold, which supports the use of a non-linear risk estimation approach.

There is broad scientific consensus that TCDD’s MoA is mediated by receptor interaction (specifically AhR), and leads to a cascading array of molecular changes that in some cases results in tumor formation—provided that the dose rate and period of exposure are sufficient. Receptor-based toxicity—including carcinogenesis—has been recognized for decades as having non-linear dose-response relationships with biological thresholds. Complementing those numerous findings are those that demonstrate that TCDD acts not as an initiator but rather as a classic promoter, a mode of carcinogenic action with characteristics that include threshold doses below which tumors are not elicited.

Utilizing this biological understanding of rodent carcinogenicity provides an important basis to develop and apply models that consolidate information to predict the shape of the dose-response curve at lower doses experienced by humans. This approach provides the basis to develop for TCDD a human risk assessment that is scientifically supported.

Our analysis of the available information demonstrates that the dose-response curve is non-linear and contains a threshold region below which tumor formation is unlikely for rodents and presumably also humans. Recent evidence (the C × T paradigm) also supports non-linearity for cancer in rodents by heptachloro-dioxin (Rozman, et al., 2005). By contrast, USEPA’s draft Reassessment used a traditional default linear extrapolation tool that, by design, has no ability to find or estimate the presence of a threshold.
Based on the evidence in this article, the first of USEPA's criteria for employing a non-linear approach: “when there are sufficient data to ascertain the MoA and conclude that it [the agent] is not linear at low doses,” appears to have been amply satisfied as is the second part of the criteria that the agent is not mutagenic. TCDD is not unique in having its dose-response function defined as “nonlinear.”

The Agency’s final Cancer Guidelines are quite clear and specific about the procedure to be used in extrapolating from high to low doses with convincing evidence of non-linearity (pp. 3–24):

For cases where the tumors arise through nonlinear mode of action, an oral reference dose or an inhalation reference concentration, or both, should be developed in accordance with EPA's established practice for developing such values, . . .

USEPA reference values are developed by selecting an appropriate and experimentally based no-observed-adverse-effect level (NOAEL) and adjusting it with several uncertainty factors. The resulting reference value is one below which adverse health consequences in humans are unlikely to occur, in effect a biological threshold.

Based on all considerations, a non-linear, threshold dose-response relationship is not only the most appropriate but also best scientifically supported to estimate human cancer risk from TCDD exposures. The observed threshold in rats lies somewhere between 22 and 28 ng/kg (5 days/week) nominal dose rate. While proof of threshold is not, and can never be absolute, the level of certainty in the case of TCDD is high because of the concordance of many lines of evidence and the consistency of repeated observations.

In selecting a linear model, USEPA has made a policy choice contrary to the strongest scientific evidence, as stated by Kroes et al. (2004, 68): “The risk estimates are based on a linearized low-dose extrapolation, which would not be appropriate for compounds such as steroids and TCDD which act via non-genotoxic mechanisms.”

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