ANALYSIS OF THE SIGNIFICANT HUMAN HEALTH RISKS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

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TCDD is a chemical for which many toxic effects have been identified, including both cancer and non-cancer effects. Although both types of endpoints are discussed here, non cancer endpoints are the focus of this evaluation.

There is a great deal of evidence in animals that TCDD is a carcinogen and mechanistic understanding strengthens the plausibility that these effects will occur in humans. The evidence for cancer in animals is definitive. Cancer has been observed at low doses in several rodent bioassays. Tumors have been consistently observed in the liver. Other reports demonstrated increased cancers in the lung, adrenal cortex, thyroid, skin, tongue and nasal turbinates (NTP, 1982, Guff et al., 1994). However, epidemiology has not provided strong evidence for cancer in humans. In highly exposed industrial populations weak associations were found between TCDD exposure and cancer with relative risks ranging from 0.0 to 3.3 (U.S. EPA 1994). Only the evidence for soft tissue sarcoma with a relative risk 3.3 was marginally significant (Fingerhut et al. 1991).

In a highly exposed population in Seveso, Italy, associations between TCDD exposure and decreases in breast cancer, which are consistent with TCDD's known mechanism of action, were observed (Bertazzi et at., 1994; Devito et al., 1992). Cancer increases reported in Seveso, Italy were not observed in the highest exposure group weakening the association (Bertazzi et al., 1994). Although there is strong evidence to suggest that TCDD is a carcinogen in animals evidence that links TCDD to human cancers is equivocal.

TCDD is not genotoxic, however it has been reported that TCDD disrupts pathways of cell growth and differentiation. Specifically, it has been suggested that growth factors such as epidermal growth factor, transforming growth factor α, interleukin 1β, estrogen receptor, glucocorticoid receptor, plasminogen inactivating factor, and gastrin have been altered after administration of TCDD (Lucier et al., 1993). This disruption may also lead to altered growth regulation and be the pathway to tumor promotion.

Additional evidence for tumor promotion is found in the understanding of the biochemical changes associated with TCDD administration. The effects of TCDD are receptor mediated. Upon entering the cell TCDD binds to the high affinity Aryl hydrocarbon (Ah) receptor. Its toxicity is generally dependent on the binding to this cytosolic receptor. Subsequently the receptor-TCDD complex may bind to elements on the DNA and cause enhanced transcription of proteins. These proteins include growth factors that can inhibit or enhance growth and

differentiation of cells. The changes in growth factors observed after TCDD administration are consistent with the mechanism of toxicity.

In a comparative analysis of the tissue level of TCDD at which cancer and non-cancer effects occurred, liver cancer (increased hepatic hyperplastic nodules) was observed at concentrations of 5100 ppt (Kociba et al., 1978). Non-cancer effects in rodents occurred at concentrations ranging from 0.029 to 806 ppt. Based partly on the analysis it was concluded that non-cancer endpoints were at least as important if not more significant in the assessment of health risks of TCDD (Table 1).

Table 1 Summary LOEL Non-Cancer Endpoints vs. Cancer Endpoints

Endpoint	LOEL - Tissue dose - liver ppt	Species	Reference
increased hepatic hyperplastic nodules	5100 ppt	Sprague-Dawley rat	2 year Rodent Bioassay - Kociba et al., 1978
reduction in spermatogenesis	806 ppt	Sprague-Dawley rats	Mably et al., 1992 rats
increase Cyp1A1/Cyp1A2 initiation-promotion	480 ppt	female Sprague-Dawley rats	Tritscher et al., 1992
20% decrease in CD4+ lymphocytes	70 ppt	marmosets	Neubert et al., 1990
increase EROD	10.2 ppt	female Wistar rats	Abraham et al., 1988
increase benzo(a)pyrene hydroxylase (AHH)	5.4 ppt	female Sprague-Dawley rats	Kitchin and Woods, 1979
decrease splenic plaque forming cells	0.084 ppt	B6C3F1 female mice	Narasimhan et al., 1994
increase protein tyrosyl phosphorylation	0.084 ppt	B6C3F1 female mice	Ma et al., 1992 rats

[a] value based on estimate from Rose et al., 1976

Administration of TCDD has consistently been shown to cause a variety of toxic effects. In man, exposure to TCDD has resulted in chloracne. In monkeys there is hair loss along with thickening and keratinization of glandular tissue. Oral administration in monkeys, rabbits, and hairless mice results in dermal lesions. Gastrointestinal lesions characterized as hyperplasia of the gastric mucous or intestinal epithelium have been found in monkeys, cows

and hamsters but not in rats, and guinea pigs. Urinary tract hyperplasia has been reported in the guinea pig, monkey and cow. Liver toxicity, immunotoxicity, and thymic atrophy occur in several species at very low doses (1-55 ng TCDD/kg). In addition, tissue specific fetal development is affected by TCDD in several species (Pohjanvirta and Tusmisto, 1994). Presented here is the quantitative comparison of dose response for several non-cancer endpoints.

To evaluate the dose-response of TCDD and ultimately recommend reference doses (RfDs), many TCDD studies that resulted in adverse non-cancer effects were reviewed. Data in the published literature was the main source of this information. Studies with multiple dose points (>2) were selected for further dose-response analysis. Studies demonstrating frank health effects or lethality were excluded. Based on this screen there was sufficient data to evaluate three endpoints; immunological, biochemical and developmental. The tissue-dose evaluation of developmental effects, particularly cleft palate, was hindered primarily because there was little information about tissue distribution to the fetus. In vitro cultures of human, rat or mice palate tissue had also indicated that the human abnormality of cleft palate does occur, but at orders of magnitude higher than in mice or rats. It was determined that biochemical and immunological data had enough reports in a low dose range to warrant further analysis.

Currently there are two approaches to set acceptable risk levels for non-cancer endpoints: the No-Observed-Adverse-Effect-Level (NOAEL)/Safety Factor approach and the Benchmark Dose approach. Neither method incorporates an understanding of the biology of the toxic response. However, for TCDD there is a great deal of mechanistic understanding. The method described here attempted to incorporate mechanistic understanding into the empirical analysis of non-cancer effects which will add confidence to acceptable dose estimates.

Dose-response analysis was first performed using the Sigmoid-Emax (EMAX) function. This function was derived from Hill kinetics, a well accepted model for receptor binding, which models initiation of receptor binding and the receptor saturation phenomenon (Holford and Scheiner, 1981).

$$y + E_0 + \frac{(ax^g)}{(b^g + x^g)}$$

Sigmoid Emax for increasing responses

$$y = E_{max} - \frac{(ax^g)}{(b^g + x^g)}$$

Sigmoid Emax for decreasing response

(x = dose; y = response; E_0 = effect at zero dose; Emax = maximum response reported; a = maximum response - estimated; g = kinetic order; c = concentration of x at 1/2 maximum response = EC_{50})

Additionally, the Power Law function, a subset of the EMAX function, and the Linear Function were applied to these data. Each of these functions were applied to mean and standard deviation data using non-linear regression analysis to estimate slopes. Goodness of fit was measures using R^2 and estimates of standard error.

In this dose response analysis, the immunological and biochemical data were fitted to the EMAX function using non-linear regression analysis (McGrath et al., 1994). Ethoxyresorufin-o-deethylase (EROD) and benzo(a)pyrene hydroxylase (AHH) were examined as biochemical endpoints (Kitchin and Woods, 1979; Abraham et al., 1988; and Narasimhan et al., 1994). When the EMAX, Power Law and Linear Functions were applied to the biochemical dataset there was no single function that provided a better fit, and none of the slope estimates differed significantly from one.

The same analysis was also performed for the immunological endpoints (including t-dependent and t-independent plaque forming cell enumeration, and CD4+ lymphocyte cell counts). Seven studies that measured immune suppression (Kerkvliet et al., 1990; Silkworth et al., 1993; Narasimhan et al., 1994; Davis and Safe, 1988,; Harper et al., 1993; and Neubert et al., 1990) were evaluated. The EMAX function provided a clearly better fit to these data than the Linear or Power Law Functions. The slope of the EMAX function did not differ significantly for 6 or 7 studies, with slope estimates ranging from 3.2-4.1. This highlights the consistency of this endpoint and gives a clear indication that the slope of dose-response curve for TCDD mediated immunosuppression is non-linear at low doses.

After selecting the optimal curve fit, and estimating the slopes, Benchmark Dose estimates were calculated and compared. The Benchmark Dose estimates for selected immunological and biochemical endpoints are enumerated in Table 2. The ED_{10} estimates were in the administered dose range, therefore, the interpolation of data adds credibility to the estimated value.

This analysis demonstrated that there is a consistent decrease in Benchmark Dose estimates of 1-2 orders of magnitude for biochemical endpoints in addition to consistency across studies (Table II). However, the NOAEL estimate differed by an order of magnitude between studies.

The same type of analysis was performed on the immunological endpoints with markedly different results. The value of the Benchmark Dose estimates changed very little as the effect changed. Although all data are not reported here, the values remained consistent within 6

of the 7 studies. This included data from the plaque-forming cell assay in mice and CD4+ cell lymphocyte assay. In addition, all the Benchmark Dose estimates including the very lowest $ED_{0.0001}$ are higher than the value obtained using the NOAEL/Safety Factor approach.

The application of the Benchmark Dose approach to both immunological and biochemical data demonstrated that the RfD estimates are more consistent than estimates derived from the Safety Factor approach, and that the Benchmark Dose approach should be used over the NOAEL/Safety Factor approach. In addition, the effect dose estimates for biochemical and immunological endpoints changed at different rates suggesting there may be a mechanistic difference in these endpoints.

This analysis demonstrated that incorporating information on the mechanism of toxicity can improve RfD estimates. This manifests itself in the selection of a dose response function, selection of critical effects, and applicability of animal data to human data.

Using low dose data (data approaching environmental concentrations) for the empirical analysis provided information that is relevant to human health risk assessments. For immune suppression, the evaluation of multiple studies demonstrated the non-linearity of this endpoint which is incorporated into the Benchmark Dose approach. In contrast, the analysis of biochemical endpoints suggests linearity at low doses, a finding that is also reflected in the Benchmark Dose estimates. The selection of a critical endpoint for human health risk assessment should not be dependent on the linearity of non-linearity of the endpoint at low doses, but on the relevance of the endpoint to human health. For immune suppression, there is a clear relationship with frank health effects at high doses. For biochemical data the ultimate health effect is less clear. Immune system suppression is more clearly defined as an adverse health outcome, is empirically consistent across several species and studies, and has been evaluated at concentrations close to environmental levels. Therefore, this endpoint should be used as the critical endpoint in TCDD risk assessment of non-cancer endpoints.

For many studies there is insufficient data to do elaborate dose-response analysis. However, as the amount of information about toxicity and metabolism of a chemical increases so should the number of options in risk assessment.

The empirical analysis of non-cancer endpoints presented here allows for a comparison of these endpoints directly to cancer values. This a paradigm shift for the EPA which will allow better monitoring of toxic chemicals in the environment.

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