United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA/600/6-88/007Ab
June 1988
External Review Draft

Research and Development

EPA

A Cancer Risk-Specific Dose Estimate for 2,3,7,8-TCDD

Review Draft

(Do Not Cite or Quote)

Appendices A Through F

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

(Do Not Cite or Quote)

EPA/600/6-88/007Ab
June 1988
External Review Draft

A Cancer Risk-Specific Dose Estimate for 2,3,7,8-TCDD

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

March 1988
Review Draft

APPENDIX A

QUANTITATIVE IMPLICATIONS OF THE USE OF DIFFERENT EXTRAPOLATION PROCEDURES FOR LOW-DOSE CANCER RISK ESTIMATES FROM EXPOSURE TO 2,3,7,8-TCDD

Steven P. Bayard, Ph.D.
Carcinogen Assessment Group

Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

CONTENTS

CONTENTS (continued)

I. INTRODUCTION AND DEFINITION OF TERMS

A. INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is the most potent animal carcinogen ever tested. It is 50 times more potent than aflatoxin B1 on a per mole basis and 50 million times more potent than vinyl chloride. In addition to its carcinogenic potency, 2,3,7,8-TCDD is also the most potent animal teratogen known and it causes other reproductive and immune system effects at extremely low doses as well.

Because of these severe toxicities, many U.S. federal and state, as well as foreign, regulatory and health agencies have proposed or implemented regulations or advisories based on levels of concern for 2,3,7,8-TCDD. Among these agencies, the U.S. Environmental Protection Agency (EPA) was, to this writer's knowledge, the first to actually produce an (upper-limit) estimate of cancer risk for 2,3,7,8-TCDD exposure to humans (U.S. EPA, 1980). This estimate was based on a methodology that extrapolated from cancer responses at doses of 1, 10, and 100 ng/kg-day in an animal lifetime feeding study (Kociba et al., 1978; Table 1) to humans at still lower levels. Initially, this extrapolation was based on a simple linear extrapolation from the lowest dose to show a significant elevated response (10 ng/kg-day caused a statistically significant increase in liver tumors--hyperplastic nodules--versus control). Shortly afterward, however, the methodology was modified to include all dose-response points in the extrapolation procedure. The new methodology was based on a multistage model for carcinogenesis due to a specific time ordering of changes, first proposed by Armitage and Doll (1954) but modified by Crump et al. (1977, 1979) to include dose-response for extrapolation purposes. Often called the linearized multistage model, the Crump model is distinguished by its approach of providing upper-limit

TABLE 1. 2,3,7,8-TCDD 2-YEAR ORAL RAT STUDY (1978) USING KOCIBA'S HISTOPATHOLOGY ANALYSIS (FEMALE SPRAGUE-DAWLEY RATS - SPARTAN SUBSTRAIN[a]) AND ELIMINATING ANIMALS THAT DIED DURING THE FIRST YEAR

Tissue and diagnosis	Dose level (ug/kg-day)			
	0 (Control)	0.001	0.01	0.1
Kociba analysis				
1. Lung Keratinizing squamous cell carcinoma	0/85 (0%)	0/48 (0%)	0/48 (0%)	7/40 (18%) ($p=2.3\times10^{-4}$)[b]
2. Nasal turbinates/hard palate Stratified squamous cell carcinoma (revised diagnosis 2/19/79)	1/54 (2%)	0/30 (0%)	1/27 (4%)	5/24 (21%) ($p=9.46\times10^{-3}$)[b]
3. Liver Hepatocellular hyperplastic nodules/hepatocellular carcinoma	9/85 (11%)	3/48 (6%)	18/48 (38%) (2 had both) ($p=3.1\times10^{-4}$)[b]	34/40 (82%) ($p<10^{-8}$)[b]
Total combined (1, 2, or 3 above) (each rat had at least one tumor)	9/85 (11%)	3/48 (6%)	18/48 (38%) ($p=3.1\times10^{-8}$)[b]	34/40 (82%) ($p=<10^{-8}$)[b]

[a]Average body weight of a female rat is 450 g.

[b]Fisher Exact Test (one-tailed).

TABLE 1. (continued)

Tissue and diagnosis	Dose level (ug/kg-day) 0 (Control)	0.001	0.01	0.1
Squire's review				
1. Lung Squamous cell carcinoma	0/85 (0%)	0/48 (0%)	0/48 (0%)	8/40 (20%) ($p=6.5\times10^{-5}$)[b]
2. Nasal turbinates/hard palate Squamous cell carcinoma	0/54 (0%)	0/30 (0%)	1/27 (4%)	5/22 (23%) ($p=1.4\times10^{-3}$)[b]
3. Liver Neoplastic nodules/hepatocellular carcinoma	16/85 (19%)	8/48 (16%)	27/48 (56%) ($p=1.3\times10^{-5}$)[b]	33/40 (82%) ($p=10^{-8}$)[b]
Total combined (1, 2, or 3 above) (each rat had at least one tumor)	16/85 (19%)	8/48 (16%)	27/48 (56%) ($p=1.3\times10^{-5}$)[b]	34/40 (82%) ($p<10^{-8}$)[b]

[a]Average body weight of a female rat is 450 g.

[b]Fisher Exact Test (one-tailed).

estimates of risk consistent with nonthreshold low-dose linearity. This model is presented in section II.A.

Following EPA's efforts, two other U.S. agencies, the Food and Drug Administration (FDA, 1983) and the Centers for Disease Control (CDC) (Kimbrough et al., 1984) produced cancer risk estimates based on slight modifications of EPA's methods but with similar resulting estimates. In another minor variation, the State of California (1984) used the Crump linearized multistage model to extrapolate the cancer response to humans from a mouse gavage study performed by the National Cancer Institute (NTP, 1982). All efforts produced results within a factor of 10. These are discussed in section II.B.

Within the framework of the linearized multistage model and the Kociba et al. rat feeding study, two other efforts appearing in the literature are noteworthy. First, Longstreth and Hushon (1984) applied several mathematical nonthreshold, nonlinear models (Logit, Probit, Weibull, and multihit) to the cancer response in the Kociba et al. study, and compared the extrapolated results with those of the linearized multistage model. Second, Sielken (1987) fit the Kociba data with the multistage model (but not the Crump linearized version applying upper limits) and also with a modified version which allowed the input of actual observation times. He then compared actual estimates derived from the multistage model with those of the EPA, which used the upper limits based on the Crump version. The Sielken paper is discussed further in section II.D.

In contrast to all of the above attempts at extrapolating from animal data to humans by nonthreshold models, several U.S. nonregulatory agencies have applied safety or uncertainty factors, not models. The uncertainty factors of between 100 and 1,000 are applied to doses that have shown no adverse effects in animal cancer or other studies, and the resulting numbers are presumed safe

for humans. This methodology has been used by EPA and many other agencies for animal-to-human extrapolations for toxic effects other than cancer, but EPA has never used this methodology to estimate cancer risk. The estimates of cancer risk provided by the uncertainty factor approach are in the range of 150 to 1,500 times lower than the estimates provided by EPA's use of Crump's multistage model. These estimates are presented in section III.A.

The differences in the magnitude of the estimates provided by these two approaches require a closer look at the methodologies involved in each and in the reasoning as to which is the proper one to use for cancer risk extrapolation of 2,3,7,8-TCDD. The argument focuses on the model for complete carcinogens versus promoters. Complete carcinogens have both initiating and promoting ability, and it is the mechanism leading to the initiating part of carcinogenesis, the attachment of the carcinogen to the DNA, that can be modeled on either a linear or multistage basis. Those in favor of modeling 2,3,7,8-TCDD as a complete carcinogen argue that 2,3,7,8-TCDD causes rare cancers of the hard palate and nasal turbinates, tongue, (in male rats), and a rare form of lung cancer, and that such rare tumors would be unlikely to be initiated except by the 2,3,7,8-TCDD in the experiment. Conversely, those in favor of the uncertainty factor approach point to the strong evidence for the promoting effects of 2,3,7,8-TCDD in the liver where most of the tumors are occurring. They argue that promotion is effectively a toxic reaction with a threshold and that all promoters show not only thresholds but also reversibility upon cessation of dosing. Treating 2,3,7,8-TCDD as a promoter and using an uncertainty factor approach has a further advantage of comparing its cancer effects with its other toxic effects using the same methodology.

A third approach is also possible. This is an approach which models for the cancer effects of 2,3,7,8-TCDD through its known mechanism of binding to a

receptor. (This actual modeling and results are presented in section IV.B.) While the basic model, the Moolgavkar-Venzon-Knudson (M-V-K) two-stage model, with a promotion phase, has been used in the literature to explain many cancers, and has been found to predict well the tumor promotion in mouse skin (Chu et al., 1987), the approach is new in that modeling for promoters has never been done before by regulatory agencies.

The purpose of the presentations that follow is to compare quantitatively the estimates derived from each of the separate approaches and then to compare them with each other. In order to do this, common terms must be introduced.

B. DEFINITION OF TERMS

1. Terms Associated with Modeling (defined here as an estimation of the incremental cancer risk to humans arrived at by fitting a mathematical function to animal response data)

Maximum likelihood estimate (MLE)--The statistical procedure by which the parameters of the model are estimated. The MLE has many properties, in a statistical sense, which allow it to be referred to as a "statistical average" or "best" estimate. In risk terms it might be thought of as a term that, if the assumed model is true, provides overestimates and underestimates of the true risk each 50% of the time.

Parameter--A constant in the model, associated either with the control response, or the time or dose variable inputs. For example, in the Crump linearized multistage model, the parameter associated with the linear dose variable is denoted as q_1 and is defined as the increase in cancer risk associated with an incremental increase per unit of dose. For this reason q_1 is expressed in units of reciprocal dose such as $(ng/kg\text{-}day)^{-1}$.

Upper-confidence limit (UCL) estimates--The estimates resulting from a

statistical procedure in which the upper-limit values of the parameters still consistent with the data are estimated. In the linearized multistage model (Crump), the upper-limit estimate associated with the linear term is designated q_1^*. In a statistical sense it is the 95% upper-limit estimate of the linear term associated with the fitting of the linearized multistage model to the animal data. In making cross-species extrapolations to humans, however, the "95%" label is dropped, since the uncertainty associated with cross-species extrapolations is considered far greater than the statistical uncertainty associated with the model-fitting procedure. Also, because the linearized multistage model becomes linear at low doses, the UCL on q_1^* is the same as the UCL on the incremental risk. This is not true of the nonlinear models such as the Logit, Probit, and Weibull discussed in section II. Upper-limit incremental risk estimates, however, are comparable, and the ratio of these estimates from two different models can be expressed as the relative potency.

Risk specific dose (RsD)--A dose associated with a specified cancer risk. For example, assume a linearized multistage model is fit to the data and the parameter estimates are $q_1 = 3.0 \times 10^{-3}$ $(\text{ng/kg-day})^{-1}$, $q_i = 0$ for all $i \neq 1$, and $q_1^* = 7.5 \times 10^{-3}$ $(\text{ng/kg-day})^{-1}$. Then for an incremental risk of 1 in 1,000,000, the dose would be the solution to $10^{-6} = 1-\exp(-3.0 \times 10^{-3}\ d)$, and RsD = 3.3×10^{-4} ng/kg-day would be called the risk specific dose. Likewise, the RsD could be defined in terms of the lower limit of the dose corresponding to a risk of 10^{-6}. In this case q_1^* would be substituted for q_1 and the solution would be RsD = 1.3×10^{-4} ng/kg-day.

A ratio of two RsDs can also be thought of as a measure of relative potency, but in this case the higher the RsD, the lower the potency. The RsD thus becomes a common unit to discuss relative potency between different approaches and different types of toxicity.

Virtually safe dose (VSD)--A dose associated with a very small risk. The general reasoning in discussing RsDs and VSDs is identical. The only difference is in one's definition of a "very small risk."

2. Terms Associated with the Uncertainty Factor Approach

The lowest-observed-adverse-effect-level (LOAEL) is defined as the lowest dose in an experiment at which there is a statistically significant increase over the control group in the proportion of animals for which adverse effects are observed. The no-observed-adverse-effect-level (NOAEL) and no-observed-effect-level (NOEL) are straightforward negations (Crockett and Crump, 1986). The uncertainty or safety factor is an arbitrary factor applied to these levels for the purpose of establishing concern or no-concern levels for humans.

II. EPA's USE OF THE LINEARIZED MULTISTAGE MODEL FOR CARCINOGEN RISK EXTRAPOLATION AND COMPARISON WITH OTHER MODELS

A. DESCRIPTION OF THE MULTISTAGE AND LINEARIZED MULTISTAGE MODELS

EPA's reasons for using the linearized multistage model for risk extrapolalation, in general, are discussed in the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986). For the 2,3,7,8-TCDD risk assessment, additional discussions are presented in the Health Assessment Document (HAD) for Polychlorinated-Dibenzo-p-Dioxins (U.S. EPA, 1985) as well as in the document on the cancer risk-specific dose estimate for 2,3,7,8-TCDD. Therefore, only an abbreviated review of its development will be presented here. Basically, its genesis came from Armitage and Doll who proposed a theory that a cancer cell was generated from a series of several heritable mutations in a specific order, the end result of each change being termed a stage. The transition rate from one stage to the next was hypothesized as being related to a probability of occurrence. The time

rate of occurrence of the ith event is $a_i+b_id_i$, i=1, ..., k, where a_i, $b_i \geq 0$ and d=dose. This, along with some other assumptions, leads to a dichotomous response probability of the form

$$P(d) = 1-\exp-[c \prod_{i=1}^{k} (a_i+b_id)] \qquad a_ib_i \geq 0$$

where a_i is the background transition rate for a progression to stage i, b_i is the incremental increase in that rate per unit of dose, c is a function of exposure duration, and P(d) is the probability of a tumor by some fixed age t for a dose d. This model achieved some popularity mainly because of its success at predicting many of the human epithelial cancers, and because the model now presented the probability of a tumor as a function of dose. In addition, the reparameterization of the individual transition rates leads to

$$P(d) = 1-\exp-(q_0 + q_1d + q_2d^2 + \ldots + q_kd^k)$$

where P(d) is the lifetime probability of cancer at dose d. Since q_0 is the parameter associated with the background rate, an assumption of independent background leads to

$$P_t(d) = 1-\exp-(q_1d + \ldots + q_kd^k) \qquad \text{all } q_i \geq 0$$

where $P_t(d)$ is the incremental (often called the extra) risk associated with dose d. In the linearized form of this model an upper-limit estimate of the linear term, q_1^*, consistent with the data, is calculated. At low doses, this upper-limit linear term predominates, forcing the model to low-dose linearity.

B. USE OF THE LINEARIZED MULTISTAGE MODEL FOR RISK EXTRAPOLATION OF 2,3,7,8-TCDD: COMPARISON OF FOUR U.S. AGENCIES

Besides the choice of the linearized multistage model for animal-to-human

risk extrapolation, the final risk estimates are dependent on a choice of several other factors. Specifically, Table 2 presents a summary of 2,3,7,8-TCDD cancer risk extrapolation by four U.S. agencies, all of which used the linearized multistage model. Even with the use of the same model, however, the results varied over 10-fold due to a selection of different factors relating both to the animal data and to the procedure. Such factors are:

- Choice of animal bioassay
- Adjustment made for differential nontumor mortality among the animal treatment groups
- Selection of tumor types for modeling
- Animal-to-human dose equivalence
- Dose used for curve fit

As seen in Table 2, EPA, FDA, and CDC all used the cancer response data from the female Sprague-Dawley rat in the 2-year feeding study conducted by Kociba et al. (1977, 1978). In the EPA HAD for Polychlorinated Dibenzo-p-Dioxins, the choice of the Kociba study was based on the female rat providing the largest slope factor, q_1^*, of all the available data sets. However, there were other, unstated but probably better, reasons for the selection, such as (1) the high quality of the study, (2) response at multiple sites, (3) more applicable route of exposure to the human experience than the gavage study, and (4) less controversial tumor sites than the mouse liver. The State of California used the liver tumor response from the male mouse in the National Toxicology Program (1982) gavage study and estimated an upper-limit incremental unit risk estimate of $q_1^* = 1.5 \times 10^{-7}$ $(fg/kg\text{-}day)^{-1}$, nearly the same as that of EPA ($q_1^* = 1.56 \times 10^{-7}$). Also, in its analysis EPA contracted with an independent pathologist, Dr. Robert Squire, to provide a second examination of the liver slides in the Kociba study. Even though Squire's analysis indicated more liver

TABLE 2. FACTORS USED BY VARIOUS AGENCIES IN CALCULATING THEIR UPPER-LIMIT RISK ESTIMATES FOR 2,3,7,8-TCDD USING THE LINEARIZED MULTISTAGE MODEL

Factor	EPA	FDA	CDC	State of California	Effect of difference on upper-limit unit risk estimate
Animal study used	Kociba female rat feeding study	Kociba	Kociba	NTP male mouse mouse gavage study	Based on dose/surface area dose conversion difference is less than 10%
Pathologist (Kociba or Squire)	Both	Kociba	Squire	NA	Less than 10%
Adjustment for high early mortality in high-dose group	Yes	No	No	NA	Adjustment changes estimate by a factor of +1.7 or 1/2.6[a]
Selection of tumor types	Liver, lung hard palate/ nasal turbinates	Liver only	Liver only	Liver only	Less than 10%
Animal-to-man dose equivalence	$\frac{\text{dose}}{\text{surface area}}$	$\frac{\text{dose}}{\text{body weight}}$	Liver concentration	$\frac{\text{dose}}{\text{surface area}}$	Dose/surface increases estimate by a factor of 5.38 over the other two for the rat, by a factor of 13 for the mouse
Dose used for curve fit	Administered	Administered	Liver concentration at terminal sacrifice	Administered	Using administered dose decreases estimate by a factor of 2
Upper-limit incremental unit $(fg/kg\text{-}day)^{-1}$	1.56×10^{-7}	1.75×10^{-8}	3.5×10^{-8} (when reconverted to administered dose)	1.5×10^{-7}	
Reference dose for upper-limit risk of 10^{-6}; units of fg/kg-day	6.4	57.2	27.6	6.7	

[a]Adjustment increases by a factor of about 1.7 compared with unadjusted Kociba analysis; adjustment decreases estimate by a factor of 2.6 compared with unadjusted Squire analysis where high dose is excluded due to poor fit.

tumors in the control and low- and mid-dose groups, the estimates of an incremental increase in cancer risk differed by less than 10%.

Another factor of concern in the extrapolation procedure was nontumor toxicity among the female rats. In order to correct for high early mortality in the high-dose rats, EPA's analysis eliminated all animals that died during the first year of the study (before the appearance of the first tumor). The elimination of nine animals in the high-dose group, one in the controls, and one in each of the other dose groups, changed the upper-limit estimates by a factor of either +1.7 or 1/2.5 depending on which pathologist's analysis was used. Since EPA was the only agency to make the adjustment, its estimate incorporating both pathologists' analyses actually decreased by a factor of 2.7 compared with the unadjusted analysis.

In selection of tumor types, all the agencies modeled the liver tumor response. EPA also included the cancer response in the lung and hard palate/nasal turbinates, but this led to only a minor increase in the final estimate since the liver produced the major response.

Animal-to-man dose equivalence factors are discussed in the HAD. Both EPA and the State of California used dose/surface area equivalences between animal and humans. The FDA used dose/body weight which reduces human risk estimates compared to surface area by a factor of 5.4 for rat-to-human extrapolation. The CDC used liver concentration at terminal sacrifice, a measure that would be preferable if human tissue distribution was also known. In the present case, however, the known rat liver concentration measures of dose equivalence had to be equated back to the rat administered dose without a comparable known relationship in humans. The dose used for the curve fit by EPA, FDA, and the State of California was the dose actually administered to the animals. The CDC's use of liver concentrations at terminal sacrifice resulted in the risk

estimate being increased by a factor of 2. Species conversion factors are discussed further in section II.C.

The end result of all of these factors in the risk extrapolation procedure resulted in a maximum difference of a factor of 9, that being between the EPA and the FDA. This can be seen either by comparing the upper-limit incremental unit risk estimates or the risk specific doses (RsDs), which are just the reciprocals $x10^{-6}$.

C. ALLOMETRIC AND BODY BURDEN CONSIDERATIONS

The dose metric or allometric equivalence for rat-to-human extrapolation has a potentially large quantitative impact on 2,3,7,8-TCDD risk estimation because of the large differences in half-lives in the rat and human. However, what little attention this topic has received from regulatory agencies until now has taken into account only the standard dose metrics. As shown in Table 2, both EPA and the State of California used the administered dose/surface area conversion, the FDA applied an administered dose/body weight and the CDC applied the actual rat liver concentration at terminal sacrifice. When extrapolating from rat to human, use of the dose/surface area allometry increases the risk estimate by a factor of 5.4 versus either dose/body weight or liver concentration. When extrapolating from the smaller mouse to human, the corresponding use of dose/surface area allometry results in a factor of 13 greater risk estimate.

The EPA has used the dose/surface area metric, often called a species extrapolation correction factor, as a conservative, prudent policy. It is based on the observations that among different mammalian species many physiological rates, and especially ventilation, basal metabolic, and clearance rates tend to scale in proportion to a fractional power of body weight. It has also been found

to hold for the acute therapeutic effects of anticancer agents. That fractional power, often between 0.6 and 0.8 is very close to the fractional power of 2/3 relating the surface area of cylindrical or round objects to their volume. Since the density of most mammalian bodies is about the same, mass or body weight can be used instead of volume, and hence the term surface area or (body weight)$^{2/3}$ correction. In simplified terms the allometry of basal metabolism is often explained by the observation that the amount of calories a warm blooded animal will consume is enough to maintain body temperature and that loss of heat is related to surface area and not mass. However, the allometry of species conversion for carcinogen risk assessment is far more complicated than simple basal metabolism. Even assuming that the basic mechanism of the carcinogen stays the same from high- to low doses, there are often large species differences both in tissue distribution and in metabolic pathways to form the active carcinogen. Often, it is not known whether the parent compound or one (or more) of its metabolites is the active carcinogen. Almost never is there a good understanding of the mechanism.

It is just because of these many unknowns that regulatory agencies have been forced to adopt a general default position of a surface area or body weight or parts per million (ppm) in air species conversion factor. The EPA most often uses surface area, but sometimes uses ppm in air as a species dose equivalence, based on the known cross-species allometry for O_2 consumption. The FDA position is to use dose/surface area allometry when the active carcinogen is a metabolite of the administered compound and to use dose/body weight when the active carcinogen is thought to be the administered compound itself. Their reasoning for the latter case, of which they consider 2,3,7,8-TCDD an example, is that if the administered compound does not have to be metabolized to be carcinogenic, then strict dose/body weight considerations should apply. The

CDC apparently agrees. The EPA counters, however, that even if the parent compound is the active carcinogen, its activity is related to its time in the body, which in turn is related to clearance time and hence to dose/surface area allometry.

At this point, it may be instructive to derive the dose/surface area allometry in a slightly more rigorous manner. The rat-to-human species correction factor of 5.4 means that if the human were to receive the dose/body weight of a compound, 5.4 times that of the rat, the different elimination capacities of the two species would cause both species' concentration x time exposures to the compound to be equal. In terms of first-order elimination kinetics for a single dose of a nonmetabolized compound, a rat given concentration C_0 with an elimination constant k_e would have a total area under the concentration-time curve of C_0/k_e. A human given a concentration of $C_0/5.4$ would eliminate the material, if allometric considerations hold, at a rate of $k_e/5.4$, so that his total area under the concentration-time curve would be equal to that of the rat.

For continuous daily exposure, the total area under the concentration-time curve is (C/k^2) $(TK - 1 + e^{-kT})$, where C is the daily dose/body weight and T and k are units of days and reciprocal days, respectively. If T is large, say 730 days for a 2-year rat study and k is not very small, then this area becomes approximately CT/k. Thus, in order for the total areas to be the same for a 2-year rat and 70-year human dosing period, the human would have to be given a concentration C/(5.4x35) or 1/189 that of the rat. EPA's position in this metric is that one rat year is equivalent to 35 human years in the cancer development process, and that the cancer age-distributions for rats and humans are alike when T is viewed as representing a lifetime. Therefore, over a lifetime a human should be allowed 35 times the C/k that of the rat as an equivalent dose.

Since rats and humans seem to follow closely enough for most compounds,

the clearance rate dose allometry discussed above, adjusting the species correction factor for actual clearance rates, is not often done in risk extrapolation. Furthermore, consideration of all the unknowns of actual mechanism and metabolism make it apparent that the species correction factor is only a rough approximation, meant to somehow convey the concept of increased sensitivity of the human compared to the smaller animals. Still, the appropriateness of the use of the surface area correction factor is not clear in the case of 2,3,7,8-TCDD because of its extremely long half-life in humans compared to rats. The potential effect of this difference on quantitative risk estimation is now examined.

Rose et al. (1976), examined the fate of 2,3,7,8-TCDD following single and repeated oral doses to Sprague-Dawley rats. For a single oral dose of 1.0 ug TCDD/kg body weight, they found a half-life, assuming a one compartment open model, of 31 $\pm$ 6 days. For repeated oral doses of 0.01, 0.1, or 1.0 ug TCDD/kg-day, 5 days a week for 7 weeks, they found a half-life of 23.7 days. For the single dose, after 22 days nearly all of the compound had concentrated in either the fat or the liver, with equal concentrations in each. For the rats administered the repeated oral doses, the compound again concentrated mostly in the liver and fat, with the liver concentration being three to five times as high as that of the fat at the end of 7 weeks. This observation is consistent with that of Kociba et al. (1978) who, in his 2-year feeding study, found liver concentrations three to five times as high as adipose tissue when the daily dose was at least 0.01 ug/kg-day and about the same as the adipose tissue concentration when the daily intake was 0.001 ug/kg-day. Rose et al. estimated the elimination constants for liver, fat, and whole body all about equal, 0.026 $days^{-1}$, 0.029 $days^{-1}$, and 0.029 $days^{-1}$, respectively, corresponding to half-lives of 24 to 27 days. The relationship between half-life and clearance times for first-order kinetics is $t_{1/2} = \ln 2/k_e$.

In humans the half-life of 2,3,7,8-TCDD in the body has been variously estimated as 3 to 5 years, 6 years, 10 years, and if 2,3,7,8-TCDD acts according to two compartment kinetics with the fat acting as a "deep" second compartment, up to 30 years (U.S. EPA, 1988). The CDC (1987) estimates a half-life of 6 to 10 years; this estimate will be used here.

An additional complication is that nonhuman primates, unlike rats, apparently accumulate a higher concentration of 2,3,7,8-TCDD in the adipose tissue than in the liver, with ratios ranging from 10:1 to 67:1. The very limited human data also suggest an adipose tissue to liver concentration ratio of 10:1, with minor deposition in other organs. One experiment exposing rats and both infant and adult monkeys to a single intraperitoneal injection (400 ug TCDD/kg body weight) found that after 7 days the rat had concentrated 43% of the administered dose in its liver versus only 10% and 4.5% for adult and infant monkeys. In monkeys, the larger percentages were found in adipose tissue (U.S. EPA, 1985). Thus, if the liver is the organ of primary concern, for tissue distributions alone a human would have to be given anywhere from 10 to 50 times the dose on a mg/kg-body weight basis to have the same liver concentrations as the rat. If one is not concerned with the liver alone but with total body burden, it is not these figures but the relative body half-lives which would apply.

Estimates of the ratio of half-lives in the human versus the rat show that for a human half-life of 2,190 to 3,650 days (6 to 10 years) and a rat half-life of approximately 25 days, the ratios are 88:1 to 146:1, far higher than the 5.4:1 correction used by EPA. If liver is the focus and comparative liver tissue distributions are factored in, however, the rat-to-human correction factor ranges from 1.8(88/50) to 36.5(146/4).

The quantitative risk implications of these adjustments for tissue distribution and half-life differences between the rat and the human are presented

in Table 3. As can be seen, if total body burden (area under the time-concentration curve) only is considered, the very long half-life in the human leads to risk estimates between 16 and 27 times that in EPA's HAD (1985). If liver concentrations are considered however, the relative risks range from 0.3 to 6.8 times that of EPA's estimate. All estimates are higher than the FDA's. The limited evidence suggests that if liver tissue concentration-time species equivalence is correct, then the EPA HAD (1985) might underpredict the upper-limit risk by a factor of 1.6 to 6.8.

D. OTHER EXTRAPOLATION MODELS

1. Longstreth and Hushon (1984)

Alternative models have been used for extrapolating to low-dose risk. Three of these, the one-hit, the Probit, and the Weibull, have been discussed and modeled in Appendix C of the HAD (U.S. EPA, 1985). The latter two, plus two others, the Logit and the multihit, have been modeled by Longstreth and Hushon (1984), but they have not adjusted their data for high early nontumor-related mortality. This has been done in Tables 4 and 5 for the Kociba and Squire histopathology analyses, respectively. The resulting MLE and upper-limit risk estimates for several low-dose levels are consistent for both data sets.

For both data sets the linearized multistage and one-hit models yielded identical results. Also, for both data sets the upper-limit estimates based on the multistage model were consistent, while those based on the other three models varied considerably. For example, at a dose level of 10^{-5} ng/kg-day the UCLs for the multistage model were 1.5×10^{-6} and 1.6×10^{-6} for the Kociba and Squire pathology analyses, respectively. However, at the same dose level of 10^{-5} ng/kg-day, the UCLs for the Logit model varied by a factor of 29, from 2.0×10^{-6} for the Kociba pathology to 5.8×10^{-5} for the Squire pathology.

TABLE 3. RISK EXTRAPOLATIONS FOR 2,3,7,8-TCDD USING THE LINEARIZED MULTISTAGE MODEL AND VARIOUS ESTIMATES OF RAT AND HUMAN HALF-LIVES AND TISSUE DISTRIBUTIONS

Half-life (days)		Relative liver:fat tissue concentration at low doses		Calculated rat to human correction factor	Incremental risk estimates $q_1^*(pg/kg\text{-}day)^{-1}$	Potency estimates relative to EPA dose metric
Human	Rat	Human	Rat			
2190	25	Total body burden		87.6	2.5×10^{-3}	16.2
2190	25	1:4	1:1	21.9	6.4×10^{-4}	4.1[a]
2190	25	1:10	1:1	8.8	2.5×10^{-4}	1.6[a]
2190	25	1:4	5:1	4.4	1.2×10^{-4}	0.8
2190	25	1:10	5:1	1.8	4.7×10^{-5}	0.3
3650	25	Total body burden		146.0	4.2×10^{-4}	27.0
3650	25	1:4	1:1	36.5	1.1×10^{-3}	6.8[a]
3650	25	1:10	1:1	14.6	4.2×10^{-4}	2.7[a]
3650	25	1:4	5:1	7.3	2.2×10^{-4}	1.4
3650	25	1:10	5:1	2.9	7.8×10^{-5}	0.5
EPA: surface area correction				5.4	1.56×10^{-4}	1.0
FDA: mg/kg bw-day equivalence				1.0	1.75×10^{-5}	0.2

[a]Considered more likely scenarios.

TABLE 4. ESTIMATES OF LOW-DOSE RISK TO HUMANS EXPOSED TO 2,3,7,8-TCDD
BASED ON FEMALE SPRAGUE-DAWLEY RATS
FROM THE DOW CHEMICAL CO. FEEDING STUDY
DERIVED FROM FOUR DIFFERENT MODELS
(DATA: KOCIBA ANALYSIS, ADJUSTING FOR EARLY MORTALITY)

	Maximum likelihood estimates of extra risks				Upper confidence limit of additional risks			
Dose (mg/kg-day)	Multi-stage model/ One-hit model[a]	Weibull model[b]	Log-probit model	Logit model	Multi-stage model/ One-hit model	Weibull model	Log probit model	Logit model
10^{-5}	1.1×10^{-6}	1.8×10^{-5}	0	3.1×10^{-7}	1.5×10^{-6}	9.7×10^{-5}	7.7×10^{-18}	2.0×10^{-6}
10^{-4}	1.1×10^{-5}	1.1×10^{-4}	1.2×10^{-13}	4.5×10^{-6}	1.5×10^{-5}	5.3×10^{-4}	3.0×10^{-12}	2.5×10^{-5}
10^{-3}	1.1×10^{-4}	7.1×10^{-4}	4.9×10^{-9}	6.5×10^{-5}	1.5×10^{-4}	2.9×10^{-3}	7.5×10^{-8}	3.0×10^{-4}
10^{-2}	1.1×10^{-3}	4.5×10^{-3}	1.7×10^{-5}	9.4×10^{-4}	1.5×10^{-3}	1.5×10^{-2}	1.5×10^{-4}	3.4×10^{-3}
10^{-1}	1.1×10^{-2}	2.9×10^{-2}	5.2×10^{-3}	1.3×10^{-2}	1.5×10^{-2}	7.2×10^{-2}	2.3×10^{-2}	3.6×10^{-2}
1	1.1×10^{-1}	1.7×10^{-1}	1.7×10^{-1}	1.7×10^{-1}	1.5×10^{-1}	3.0×10^{-1}	3.1×10^{-1}	2.8×10^{-1}

[a]Both models gave identical results.
[b]The value of k, determined by best fit to the data, is < 1.

Human equivalent dose (ug/kg-day): 0 0.186 1.86 18.6

Animal tumors/number examined: 9/85 3/48 18/48 34/40

Human equivalence conversion: 1 ug/kg-day(oral) = 25.0 ug/m^3 in air.

TABLE 5. ESTIMATES OF LOW-DOSE RISK TO HUMANS EXPOSED TO 2,3,7,8-TCDD
BASED ON FEMALE SPRAGUE-DAWLEY RATS
FROM THE DOW CHEMICAL CO. FEEDING STUDY
DERIVED FROM FOUR DIFFERENT MODELS
(DATA: SQUIRE ANALYSIS, ADJUSTING FOR EARLY MORTALITY)

Dose (mg/kg-day)	Maximum likelihood estimates of extra risks				Upper confidence limit of additional risks			
	Multi-stage model/ One-hit model[a]	Weibull model[b]	Log-probit model	Logit model	Multi-stage model/ One-hit model	Weibull model	Log probit model	Logit model
10^{-5}	1.1×10^{-6}	3.0×10^{-4}	2.2×10^{-12}	1.0×10^{-5}	1.6×10^{-6}	1.3×10^{-3}	4.4×10^{-10}	5.8×10^{-5}
10^{-4}	1.1×10^{-5}	1.2×10^{-3}	7.6×10^{-9}	8.3×10^{-5}	1.6×10^{-5}	4.4×10^{-3}	1.1×10^{-7}	4.1×10^{-4}
10^{-3}	1.1×10^{-4}	4.9×10^{-3}	5.8×10^{-6}	6.9×10^{-4}	1.6×10^{-4}	1.5×10^{-2}	5.3×10^{-5}	2.9×10^{-3}
10^{-2}	1.1×10^{-3}	2.0×10^{-2}	9.2×10^{-4}	5.7×10^{-3}	1.6×10^{-3}	5.1×10^{-2}	5.8×10^{-3}	1.9×10^{-2}
10^{-1}	1.1×10^{-2}	7.8×10^{-2}	3.3×10^{-2}	4.6×10^{-2}	1.6×10^{-2}	1.6×10^{-1}	1.1×10^{-1}	1.1×10^{-1}
1	1.1×10^{-1}	2.8×10^{-1}	2.9×10^{-1}	2.9×10^{-1}	1.6×10^{-1}	4.3×10^{-1}	4.8×10^{-1}	4.8×10^{-1}

[a]Both models gave identical results.
[b]The value of k, determined by best fit to the data, is < 1.

Human equivalent dose (ug/kg-day): 0 0.186 1.86 18.6

Animal tumors/number examined: 9/85 3/48 18/48 34/40

Human equivalence conversion: 1 ug/kg-day(oral) = 25.0 ug/m^3 in air.

Also, at the same dose level, the UCL from the Weibull model varied by a factor of 13, while those based on the log Probit model varied by a factor of over 50 million. In general, the UCL risk estimates based on these models exhibit the relationship:

Weibull > Logit > linearized multistage = one-hit >> log Probit

All of these models fit the data satisfactorily in the animal experimental range, yet as can be seen from Tables 4 and 5, the estimates can vary over several orders of magnitude at lower environmental doses. The choice of the model must rely on factors other than goodness of fit.

2. Sielken (1987)

A second risk modeling analysis of the female rat liver response in the Kociba et al. 2-year feeding study has been published by Sielken (1987), whose arguments have also been reproduced by Paustenbauch et al. (1986). Sielken fits the multistage model to the data but focuses on the large difference in low-dose behavior depending on whether or not the high experimental animal dose of 0.1 ug/kg-day is included. Sielken's measure of risk is the VSD which he defines in terms of 10^{-6} lifetime incremental risk. The VSD is derived from an extrapolation model of the MLE of the multistage model and not from the upper-limit estimate. Sielken, thus, uses Crump's reparameterization of the multistage model but not Crump's linearized form.

Sielken's analysis is based on the argument that use of the multistage model with the 0.1 ug/kg-day dose-response included forces the estimate of the linear term to be positive non-zero and distorts the true shape of the dose-response curve.

In particular, he argues, even though the multistage model fits the data with the high-dose point included, "the (resulting) fitted models do NOT [his

emphasis] reflect the observed behavior at the lower experimental doses." A proper low-dose shape, he claims, is seen when the highest dose is removed. In this case, the linear term of the multistage becomes zero and the quadratic term becomes positive. Extrapolation with the quadratic curve goes rapidly to zero compared to low-dose extrapolation with a linear model.

An examination of the results derived from the type of analysis suggested by Sielken can be seen in Table 6. In this table, both the MLE and 95% lower-bound estimates of the VSD are calculated using different permutations of the Kociba female rat data (animals dying before the appearance of the first tumor have been eliminated). In every case, the model with estimated parameters fit the data satisfactorily. The first row contains the observed liver tumors for all doses and parameter estimates, while the second and lower rows omit the high-dose group. The third through the seventh rows permutate the low-dose data by increments of one tumor-bearing animal. The proportion of 6/48 represents the 95% upper-limit on the observed proportion of 3 tumor-bearing animals out of 48.

An examination of Table 6 shows the instability of the MLEs under this model. As was pointed out above, omitting the high-dose group changes the form of the model from linear to quadratic, with a corresponding 330-fold increase in the VSD from 4.8×10^{-8} to 1.6×10^{-5}. The form of the model remains quadratic until the number of tumor-bearing animals is incremented by 2. However, when the increment becomes 3, to total 6 out of 48, which is the 95% upper limit of the actual observed response, the picture again changes. When this 95% upper-limit of the observed low-dose response is fit, the model again incorporates a linear MLE, and the MLE of the VSD returns to 5.0×10^{-8}.

In contrast to the instability of the MLEs, the 95% lower limits of the VSDs remain quite stable over the range of permutations, varying by a factor of

TABLE 6. MLEs AND 95% UPPER-LIMIT ESTIMATES OF PARAMETERS AND VSDs FOR VARIOUS PERMUTATIONS[a] OF THE FEMALE RAT LIVER DATA USING THE MULTISTAGE MODEL. KOCIBA HISTOPATHOLOGY, ELIMINATING ANIMALS THAT DIED PRIOR TO FIRST TUMOR

				MLE-parameter estimates		VSD (ug/kg-day)		
0	0.001	0.01	0.1	Linear $(ug/kg\text{-}day)^{-1}$	Quadratic $(ug/kg\text{-}day)^{-2}$	MLE	95% Lower limit	Ratio
9/85	3/48	18/48	34/40	$2.1x10^{+1}$	0	$4.8x10^{-8}$	$3.6x10^{-8}$	1.3
9/85	3/48	18/48	Omitted	0	$3.7x10^{+3}$	$1.6x10^{-5}$	$2.0x10^{-8}$	800
9/85	4/48	18/48	Omitted	0	$3.7x10^{+3}$	$1.7x10^{-5}$	$1.9x10^{-8}$	900
9/85	5/48	18/48	Omitted	0	$3.6x10^{+3}$	$1.7x10^{-5}$	$1.8x10^{-8}$	950
9/85	6/48[b]	18/48	Omitted	$2.0x10^{+1}$	$1.6x10^{+3}$	$5.0x10^{-8}$	$1.8x10^{-8}$	2.8
9/85	7/48[c]	18/48	Omitted	$3.6x10^{+1}$	0	$2.8x10^{-8}$	$1.7x10^{-8}$	1.6

[a]All models fit the data sets satisfactorily.

[b]6/48 corresponds to the 95% upper-limit of the observed response of 3/48.

[c]7/48 corresponds to the 99% upper-limit of the observed response of 3/48.

2. The ratios of the VSDs to their lower limits reflect the instability of the MLEs for low-dose extrapolation with the multistage model.

Sielken's results are consistent with the findings of Portier and Hoel (1983), who concluded that for a multistage model fit of the data, the MLE of the linear term is usually multimodel, the number of modes being equal to the degree of the chosen polynomial. This instability of the MLEs from the multistage family of models has also been confirmed by several other authors; it is further seen in Table 6.

Crump (1987) provided a critical review of Sielken's analysis and observed that even if "the true shape of the dose response is a straight line connecting the background response and the response at the mid-dose," the probability of an MLE estimate of zero for the linear term in the multistage model is about 1/3. He concluded that while the data are consistent with Sielken's interpretation of a higher RsD, they are also consistent with those much lower, as displayed in the confidence limits.

It was based on these types of analyses and a consideration of the typical animal bioassay data to be fit that Crump had originally advised that an upper-limit estimate of the low-dose risk be used for his model. Under his reparameterization of the multistage model, an MLE of zero for the linear term becomes possible, and this can cause great instability in low-dose risk estimation. However, under the original development of the model, it is not possible to have higher degree polynomials without having a positive linear or first-stage term. Crump's reparameterization is necessary with quantal data in order to limit the number of parameters. In doing this, however, the MLEs can become unstable, as is seen above.

E. TIME-TO-TUMOR ANALYSES

An extension of the multistage model analysis can be conducted when time to observation of the tumors is known. This extension is modeled into the formulation of the multistage model:

$$P(d,t) = 1-\exp-(q_0 + q_1 d + q_2 d^2 + q_3 d^3 t^k)$$

where q_0, q_1, q_2, q_3, and k are parameters estimated from the data and are all constrained to be nonnegative. This model is often called the Weibull model, but is more appropriately described as the multistage Weibull, since it is multistage in dose but Weibull in time. Its generalization over the multistage model allows an estimation of the probability of cancer by a fixed age in the absence of any competing risks. Its superiority over the quantal or multistage form is in its ability to adjust for treatment group differences in nontumor-related mortality, as is seen in the Kociba study. However, the analysis requires a pathology decision as to whether the tumors of interest were fatal or incidental, a condition not available in the Kociba study pathology.

The results of a multistage Weibull model are presented in Table 7 and are compared with two quantal analyses using the linearized multistage model. In the first quantal approach no adjustment is made for the high early mortality in the high-dose group. In the second approach all animals dying before the appearance of the first liver tumor were dropped from the analysis (see section II.B). These two analyses are compared with the multistage Weibull under the extremes of either all fatal or all incidental tumors.

As can be seen from Table 7, the largest difference in risk estimates is between the unadjusted analysis and the fatal tumor for this analysis; 8.4 (for the MLE term) and 10 (for the upper limit). However, both these extremes are thought to be somewhat misrepresentative of the data, and either of the

TABLE 7. RESULTS OF VARIOUS TIME-TO-TUMOR ADJUSTMENTS FOR FEMALE RAT LIVER (NEOPLASTIC NODULES OR CARCINOMAS) AND THE MULTISTAGE MODEL[a] HUMAN EQUAVALENT DOSAGE (KOCIBA PATHOLOGY)

Model	Linear coefficient q_1 $(pg/kg\text{-}day)^{-1}$ of multistage model MLE	UCL	Power of time
Multistage model-quantal			
1. All animals, no adjustment	6.6×10^{-5}	9.0×10^{-5}	NA
2. Only animals surviving to time of first tumor	1.1×10^{-4}	1.5×10^{-4}	NA
Multistage Weibull model			
1. Incidental tumor[b]	2.1×10^{-4}	3.1×10^{-4}	11.56
2. Fatal tumor analysis[b]	6.6×10^{-4}	7.6×10^{-4}	7.07

[a]Three-stage model used for all analyses.

[b]Values shown for q_1 and q_1^* were derived results from WEIBULL82 by multiplying by t^k.

NA = not applicable.

middle two approaches is considered superior. While the present EPA analysis (see section II.B) yields the lower of these estimates by a factor of 2, this difference is considered minor.

III. TREATMENT AS A PROMOTER

Evidence for the promoting action of 2,3,7,8-TCDD in the rat liver has been well documented in the HAD (U.S. EPA, 1985). This section compares the quantitative cancer risk estimates under the assumption that 2,3,7,8-TCDD's action on the liver is one of promotion. It is shown that even when treated solely as a promoter the estimates of risk can vary greatly according to assumptions that one is prepared to make. In section III.A. the treatment of 2,3,7,8-TCDD as a classical toxicant or promoter with a threshold is presented, along with the results of Canadian and several European regulatory agencies who estimate "virtually safe" levels by applying uncertainty factors to dose levels at which no adverse effects are observed. In section III.B. the results of a new approach to modeling for promoters are discussed in which cancer response is modeled as a function of liver cell proliferation of initiated cells.

A. UNCERTAINTY FACTOR APPROACH

Several countries and the State of New York have estimated RsDs for 2,3,7,8-TCDD's potency by the application of uncertainty factors to NOELs, NOAELs, or LOAELs. The general approach is to use uncertainty factors ranging from 10 to 1,000 based on a rule-of-thumb approach (Cook and Page, 1986).

- A factor of 10 where adequate chronic human toxicity data as well as adequate chronic oral toxicity data in more than one animal species are available;

- A factor of 100 where adequate chronic animal toxicity data are available in more than one species, but human toxicity data are lacking; and
- A factor of 1,000 where limited chronic animal toxicity data are available (in only one species) or inconclusive results in more than one species.

The National Academy of Sciences Safe Drinking Water Committee was more explicit with regard to carcinogenicity studies (NAS, 1977).

"1. Valid experimental results from studies on prolonged ingestion by man with no indication of carcinogenicity.

Uncertainty Factor = 0

2. Experimental results of studies of human ingestion not available or scanty (e.g., acute exposure only). Valid results of long-term feeding studies on experimental animals or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

Uncertainty Factor = 100

3. No long-term or acute human data. Scanty results on experimental animals. No indication of carcinogenicity.

Uncertainty Factor = 1,000"

Others have sought to break down uncertainty factors into components. Weil (1972) interpreted the application of the uncertainty factor of 100 as a product of a factor of 10 to account for differential human sensitivities and the second factor of 10 to extrapolate results from animals to humans. When cancer became the end point of concern, others included a third factor of 10 to raise the total to 1,000. The uncertainty factors, the toxic end points, and the RsDs used by several agencies for the evaluation of 2,3,7,8-TCDD are presented in Table 8. The table includes results from the agencies that have used the

TABLE 8. COMPARISON OF RsDs AND POTENCY RATIO ESTIMATES FOR 2,3,7,8-TCDD OF VARIOUS U.S. AND FOREIGN REGULATORY AGENCIES

Agency	Risk specific dose RsD (pg/kg-day)	Potency ratio	Effect	Approach[a]	Animal study
EPA	6.4×10^{-3}	100	Cancer	LMS[a]	Kociba
California	6.7×10^{-3}	96	Cancer	LMS	NCI male
CDC	2.8×10^{-2}	23	Cancer	LMS	Kociba
FDA	5.7×10^{-2}	11	Cancer	LMS	Kociba
National Research Council (Canada)	6.5×10^{-2}	10	Cancer	LMS	Kociba
Germany	1.0	0.64	Cancer/reproductive effects	Uncertainty factor (1000)	Kociba and Murray
New York State	2.0	0.32	Cancer/reproductive effects	Uncertainty factor (500)	Kociba and Murray
The Netherlands	4.0	0.16	Cancer	Uncertainty factor (250)	Kociba
Health and Welfare (Canada) (also Ontario)	10.0	0.06	Cancer/reproductive effects	Uncertainty factor (100)	Kociba and Murray
Switzerland	--[b]	--	Cancer/reproductive effects	--	Kociba and Murray

[a]LMS = the linearized multistage model.

[b]Used the Kociba study to determine a NOEL of 1 ng/kg-day, but did not provide additional uncertainty factor.

linearized multistage model as a comparison to those agencies using the uncertainty factor approach. As can be seen, the RsDs generally segregate into two groups with those in the lower potency groups all using the NOEL approach. The State of New York, as well as the countries of Canada (plus the Province of Ontario), Switzerland, and Germany, consider the cancer study of Kociba to establish a NOEL at 1 ng/kg-day. The Netherlands also uses the Kociba study as its pertinent cancer study, but considers instead the 1 g/kg-day dose as a non-noxious dose due to the slight increase in liver cell changes in the female rats at this level. All the agencies, except Switzerland, chose the uncertainty factor approach to establish an RsD. Switzerland actually estimated inhalation and oral exposure and divided those exposures into the 1 ng/kg-day NOEL to determine a "safety factor" attributable to those exposures.

As can be seen in Table 8, potency ratios seem to gather in factors of 10. The RsDs for the five agencies above the dotted line span one order of magnitude as do those for the agencies below the dotted line. Separating the higher- and lower-potency groups is a factor of 15.6 (10 ÷ 0.64). The total potency range is 1,564. All but one agency uses the Kociba data.

B. MODELING AS A PROMOTER UNDER THE MOOLGAVKAR, VENZON, AND KNUDSON TWO-STAGE MODEL

A variation of the multistage model has been developed by Moolgavkar, Venzon and Knudson (M-K-V, 1979, 1981) which models cancer as a two-stage process with a promotion phase. This model has been shown to predict very well tumor promotion in the mouse skin (Chu et al., 1987). A variation of this model has been developed by Dr. Todd Thorslund to extrapolate from animals to provide human risk estimates of liver cancer deaths (see the Appendix to this paper). The model considers that the carcinogenic action of 2,3,7,8-TCDD is through its

dose-response relationship on the proliferation of initiated liver cells. By including what is known about the receptor-mediated mechanism involved, cell proliferation is itself considered a function of 2,3,7,8-TCDD's binding capacity, which can be shown to follow linear but saturable kinetics. When these parameters are factored into the model, the cumulative probability function, P(x) for dose x at a fixed time t, becomes

$$P(x) = 1\text{-}\exp\text{-}M\ [\exp G(x)t\text{-}1\text{-}G(x)t]/G^2(x)$$

$$G(x) = G(0) + [G(\infty)\text{-}G(0)][1\text{-}\exp\text{-}Vx]$$

where P(x) = the probability of a tumor with dose*,

M = the background mutation rate proportional to background tumor rate,

G(x) = the liver cell proliferation rate of initiated cells associated with dose x,

G(0) = the background liver cell proliferation rate,

G(∞) = the maximum cell proliferation rate possible, and

V = the parameter associated with the liver saturable kinetics of 2,3,7,8-TCDD-receptor binding.

Even though this model is termed a two-stage model, conceptually it is radically different from the multistage model in several respects. The multistage model, as computed by EPA, is a basic curve-fitting model with all parameters estimated from the data. The two-stage model with promotion as constructed can actually be fit without the estimation of any parameter from the cancer bioassay dose-response data. For example, M and G(0) can be estimated entirely from the control data, V can be estimated from a separate experiment measuring 2,3,7,8-TCDD uptake by the receptor, and G(∞) can be estimated by the incorporation of thymidine into the nuclei following administration of a saturation level of 2,3,7,8-TCDD. In theory, then, with the exception of the use of control animals for the estimation of background rates, all parameters would be estimated

separately and a goodness-of-fit test would be a good measure of how well the model fits the actual data.

An extension of the above distinction between the multistage model and M-V-K two-stage model is that the parameters derived from a bioassay based on one rat strain can be used to predict the response from a second strain with only the background rate M having to be estimated from the control group data of the second strain. This procedure was extended from animal-to-human extrapolation where human liver cancer death rates in the United States from 1980 were used to estimate the background human rates for M and G(0), and the values of the other parameters estimated from the rat data were used to provide human risk estimates.

In addition to modeling liver cell proliferation as a nearly linear function of 2,3,7,8-TCDD receptor binding (called here the negative exponential form of the M-V-K model), a second form of the model results when the 2,3,7,8-TCDD-induced cell proliferation rate is assumed proportional to the product of cellular 2,3,7,8-TCDD levels and the number of 2,3,7,8-TCDD receptors. This results in a model for the induced cell proliferation rate which is log-logistic in form.

Both models are used to extrapolate from the animal to human cancer response. The results are presented in Table 9 which is reproduced from the Appendix. While both forms of the M-V-K model fit the observed data quite well, and both can be justified on theoretical and some experimental ground, their use for low-dose extrapolation leads to a wide variation in risk estimates. For a lifetime daily dose of 0.1 ng/kg-day, or one order of magnitude below the animal experimental level, the estimates vary by a factor of more than 10,000. Furthermore, even though the negative exponential form of the model results in a linear low dose-response relationship, the risk estimates are 2 orders of magnitude below

TABLE 9. ESTIMATES OF LOW-DOSE INCREMENTAL RISK TO HUMANS EXPOSED TO 2,3,7,8-TCDD BASED ON FEMALE SPRAGUE-DAWLEY RATS[a]
COMPARISON OF THE TWO FORMS OF THE TWO-STAGE MODEL WITH PROMOTION WITH EPA's RISK EXTRAPOLATION USING THE LINEARIZED MULTISTAGE MODEL[a]

Dose (ng/kg-day)	Promotion[b]		Linearized multistage model EPA
	Negative exponential	Log-logistic	(Upper confidence limit)
10^{-5}	1.7×10^{-8}	--	1.6×10^{-6}
10^{-4}	1.7×10^{-7}	--	1.6×10^{-5}
10^{-3}	1.7×10^{-6}	$<10^{-13}$	1.6×10^{-4}
10^{-2}	1.7×10^{-5}	8.8×10^{-10}	1.6×10^{-3}
10^{-1}	1.8×10^{-4}	8.8×10^{-7}	1.6×10^{-2}
1	2.4×10^{-3}	9.3×10^{-4}	1.6×10^{-1}

[a]Taken from Table 11 of the Appendix.

[b]Squire's pathology analysis adjusting for early mortality.

the upper confidence limit obtained by EPA, which modeled 2,3,7,8-TCDD as a complete carcinogen. This difference between the estimates with the linearized multistage model and negative exponential form of the promoter model is due primarily to the low human background liver cancer rates compared to those of the female rat. However, these estimates pertain only to human liver cancer. In its present form, the model is target organ-specific from animals to humans.

Estimates with the negative exponential form of this promoter model might be considered as providing a conservative, on the high side, estimate of induced liver cancer risk, since cell proliferation is modeled as a linear function of dose. However, not enough research has been done on the low-dose properties of these models to characterize the variability of the low-dose risk estimates. While the upper-limit estimates should certainly be below those of the linearized multistage model, further research needs to be done before these models can be used for regulatory decision-making.

IV. COMPARISON OF ANIMAL PREDICTION WITH ACTUAL HUMAN DATA

With the exception of one study on 2,3,7,8-TCDD in Holmesburg, Pennsylvania, in 1967, all human exposure data are derived from accidental exposures of unknown quantity. In the Holmesburg study, 2,3,7,8-TCDD was topically applied to volunteer prisoners in total doses ranging from 0.4 ug to 7,500 ug. According to testimony and exhibits in EPA's 2,4,5-T cancellation hearings (Rowe, 1980), doses below 16 ug did not elicit a chloracne response, while a dose of 7,500 ug did cause chloracne in 8 out of 10 subjects. This high dose of 0.05 mL of a 1% 2,3,7,8-TCDD solution in 50/50 alcohol chloroform solvent was applied to one square inch of the backs every other day for a month and covered by a nonocclusive patch. If we can assume that the subjects' average age was 35, a 25% absorption

rate, an infinite half-life, and lifetime (70 years) average daily dose (LADD) proportionality, one can estimate an internal dose of 2,3,7,8-TCDD that caused chloracne to be in the 2 to 1,000 pg/kg body weight-day range. If we assume either a 5- or 10-year half-life with first-order elimination kinetics and with the other assumptions the same, then the chloracne causing the LADD range is unchanged at one significant digit. These figures correspond to an external oral dose of 4 to 2,000 pg/kg body weight-day.

Human cancer risk estimates based on the Kociba female rat feeding study with 55% absorption yield an upper-limit risk estimate of 1.56×10^{-4} $(pg/kg\text{-}day)^{-1}$. Multiplying this upper-limit estimate by 4 to 2,000 pg/kg-day and adjusting for the different absorption fractions yields an upper-limit lifetime incremental cancer risk of between 6×10^{-4} and 3×10^{-1} for humans developing chloracne. Only 10 of the Holmesburg prisoners were exposed to the highest dose, and follow-up is unclear. Nevertheless, even if their lifetime projected incremental cancer risks were as great as 0.3, and even if they were observed for their full remaining lifetimes, less than three additional cancers would be expected. Put another way, with three additional cancers expected, an observation of no additional cancers would not be highly unusual ($p = 0.055$). Clearly, unless specific types of cancer were to appear in these tested prisoners, no conclusions could be drawn.

On the other hand, Tschirley (1986) in his review of the 2,3,7,8-TCDD literature displays 9 cohorts of some 599 subjects who developed chloracne following 2,3,7,8-TCDD and phenoxy herbicide exposure. This is reproduced as Table 10. Of those cohorts, only the study of the 1949 accident at the Monsanto plant in Nitro, West Virginia (Zack and Suskind, 1980), and the 1953 accident at the BASF plant in Germany (Thiess et al., 1982), have sufficient latent period and other information to allow a comparison to be made between observed

TABLE 10. EXPOSURE TO 2,3,7,8-TCDD FROM INDUSTRIAL ACCIDENTS

Date	Workers exposed	Location of accident	Remarks
1949	250	Monsanto plant in Nitro, WV	122 cases of chloracne being studied; 32 deaths vs. 46.4 expected; no excess deaths from malignant neoplasms or circulatory disease
1953	75	BASF plant in Ludwigshafen	55 cases of chloracne, 42 severe; 17 deaths vs. 11 to 25 expected (four gastrointestinal cancers and two oat-cell lung cancers); most common injuries were impaired senses and liver damage
1956	?	Rhone-Poulenc plant in Grenoble	17 cases of chloracne, also elevated lipid and cholesterol levels in the blood
1963	106	NV Philips plant in Amsterdam	44 chloracne cases (42 severe) of whom 21 also had internal damage or central nervous system disturbances; eight deaths (six possible myocardial infarctions); some symptoms of fatigue
1964	61	Dow Chemical plant in Midland, MI	49 cases of chloracne; 4 vs. 7.8 expected deaths; 3 cancer deaths vs. 1.5 expected; one a soft tissue sarcoma
1965-69	78	Continuing leaks in Spolana plant near Prague	78 cases of chloracne; five deaths; many of the 50 workers studied for more than 10 years have hypertension, elevated blood levels of lipid and cholesterol, prediabetes; significant amounts of severe liver and neurologic damage

SOURCE: Tschirley, 1986.

and predicted risks. These involved 1.77 chloracne cases and either a 26- or 30-year latency period. Quantitative cancer risk estimates based on these cohort experiences are calculated below. However, the large uncertainty in the exposure estimates should be emphasized, and the assumptions used to derive these exposure estimates are necessary simplifications.

A. THIESS ET AL. (1982)

1. Description

At a factory in Ludwigshafen, Federal Republic of Germany, in 1953, during the hydrolization of 1,2,4,5-tetrachlorobenzol to 2,4,5-trichlorophenol, an accident happened exposing at least 70 persons. These 70 as well as 4 additional persons who were only exposed "for a short time during" 1954 and 1955 were included in the cohort. Of the 74 persons, 66 suffered chloracne or severe dermatitis. All 74 persons were successfully traced through 1979. Of the 74 persons in the cohort, 21 had died during the 26 years of observation, just slightly more than expected in any of five different control groups. However, there were seven cancer deaths observed versus 4.03 to 4.35 expected in these control groups. Of these seven cancers, three were stomach (ICD 151), one was colon (ICD 153), and three were lung (ICD 162). All seven occurred at least 10 years after the accident. A 10-year latent period will be assumed. The results are presented in Table 11. The control group represents the expected deaths based on the mortality rates of Rhinehessia-Palatinate 1970-75. The mortality for stomach cancer was statistically significant ($p = 0.016$), while that for lung cancer was marginally significant ($p = 0.09$).

2. Exposure Estimates*

Although there were no concurrent estimates or measurements of

*Contributions to this section were made by Drs. Jerry Blancato and Lorenz Rhomberg of the Office of Health and Environmental Assessment.

TABLE 11. OBSERVED AND EXPECTED DEATHS FROM STOMACH, COLON, AND LUNG CANCER IN THE 74 CASES WITH CHLORACNE OR SEVERE DERMATITIS FROM THE 1953 BASF PLANT IN LUDWIGSHAFEN (26-YEAR FOLLOW-UP WITH AT LEAST 10 YEARS' LATENCY)

Cause of death	ICD No.	Observed	Expected	SMR	p-value	95% Upper confidence limit based on Poisson distribution
Stomach cancer	151	3	0.52	5.76	0.016	1.15 - 16.8
Colon cancer	153	1	0.24	4.17	0.21	0.05 - 23.2
Lung cancer	162	3	1.05	2.86	0.09	0.57 - 8.3

Person-years at risk = 972.6.

2,3,7,8-TCDD exposure, Rappe (1987) reported a mean level of 100 pg/g in adipose tissue of four exposed BASF workers some 30 years after exposure. If we are willing to make several simplifying assumptions, then we can estimate a range of exposure. The assumptions are:

- The body is treated as a one-compartment open model.
- A range of half-lives is assumed to be between 5 to 30 years.
- Adipose tissue in a 70-kg human is about 20% or 14 kg.
- Absorption into the body is assumed to be between 5% and 25%. This figure is arbitrary and is chosen because there is less than 55% absorption in the rat feeding studies.

Under these assumptions the pertinent equation is:

$$C(t) = C_0 \, e^{-kt}$$

where $C(t)$ = the concentration in the fat at the time of analysis,

C_0 = the concentration in the fat immediately following absorption in 1953,

k = the total body elimination rate constant, assumed to be the sum of the elimination rate constants by various physiological processes, and

t = the time since absorption was completed, assumed to be 30 years.

Further, we note:

$$k = 0.693/t_{0.5}$$

where $t_{0.5}$ = the half-life of elimination from the body, and

$$\text{Exposure} = C_0 V/r$$

where V = the weight of the fat compartment (14 kg) and r = the absorption fraction. In this case, the weight of the fat compartment is used to calculate the dose. The other organs may be neglected because of the propensity of

2,3,7,8-TCDD to partition into the fat. For example, the concentration in the fat is about 100 times that of the blood.

Based on the above assumptions and estimates, the range of estimates of 2,3,7,8-TCDD exposure in the BASF plant accident is between 10 and 1,800 ug. Based on a LADD, these estimates range from 1.5 to 50 pg/kg body weight-day. The calculations are shown in Table 12. Clearly, this factor of 180 in the range of exposures and a factor of 30 in the range of LADDs creates significant uncertainty in the risk estimate calculations. In order to narrow the range, however, we note that in the Holmesburg study exposures below 16 ug caused no chloracne, while exposures of 7,500 ug caused 80% chloracne. Considering the probably greater absorption in the Holmesburg study, the exposure estimates in the top three rows of Table 12, showing the range of 220 to 1,800 ug, seem the most likely. We therefore adopt LADDs in the range of 6 to 50 pg/kg body weight-day. The risk estimates below will be calculated on the LADD of 50 pg/kg/body weight-day; risk estimates based on the lower end of the range would be about eight times higher.

3. Risk Estimates

Cancer risk estimates are calculated for the stomach cancer and lung cancer mortality presented in Table 11. These estimates are presented in Table 13. Two models are considered, the additive and relative risk models, which have been used in several EPA risk assessments. They are developed and more fully explained in the recent EPA update on dichloromethane (U.S. EPA, 1987). Both models require estimates of LADDs, and these have been estimated above as between 6 and 50 pg/kg body weight-day. The results show that incremental cancer risk estimates based on these human data are higher than those based on the animal data in every case. If the lower end of the range of LADDs had been used, the human estimates would have been greater still. The conclusion based

TABLE 12. ESTIMATES OF 2,3,7,8-TCDD EXPOSURE TO WORKERS AT THE BASF PLANT IN LUDWINGSHAFEN BASED ON MEAN ADIPOSE LEVELS IN FOUR SUBJECTS 30 YEARS LATER AND VARIOUS ASSUMPTIONS

Mean adipose concentration at T=30 years[a] (pg/g)	Half-life[b] $T_{0.5}$ (yr)	Absorption fraction r(0.05-0.25)	Elimination constant $k(yr^{-1})$[c]	Adipose concentration at T=0 years[d] C_0 (pg/g)	Exposure (ug)[e]	Lifetime average daily dose[f] (pg/kg bw-day)
100	5	0.05	0.139	6471	1800	50
100	5	0.25	0.139	6471	360	50
100	10	0.05	0.069	799	220	6
100	15	0.05	0.046	397	110	3
100	20	0.05	0.035	286	80	2
100	30	0.05	0.023	199	55	1
100	30	0.25	0.023	199	10	1

[a]Measured by Rappe (1987).

[b]Different half-lives are assumed.

[c] $k = 0.693/T_{0.5}$

[d] $C(t) = C_0 \exp(-30k)$

[e]Exposure = C_0 x 14,000 g/r

[f]LADD = dose per kg bw. Assumes area under time-concentration curve dose equivalence, average age at accident = 35 years. Formula is (see section II.C.): $LADD = [C_0 \cdot (1-\exp(-35K))K/70(365)K-1]\ 14/70$

TABLE 13. COMPARISON OF INCREMENTAL CANCER RISK ESTIMATES FOR LIFETIME EXPOSURE TO 2,3,7,8-TCDD BASED ON THE KOCIBA FEMALE RAT LIVER, LUNG, AND HARD PALATE/NASAL TURBINATES (HP/NT) RESPONSE WITH ESTIMATES BASED ON LUNG AND STOMACH CANCER MORTALITY IN 74 WORKERS AT THE BASF PLANT IN LUDWIGSHAFEN
(ASSUME LADD = 50 pg/kg bw-day)[a]

Species/ sex	Model used	Cancer type	Parameter estimates[b] Δ	Asymptotic variance of estimates	Lifetime incremental cancer risk per 1 ng/kg-day continuous exposure		
					Lower limit	MLE[c]	95% Upper limit[d]
Humans/ Males	Additive	Stomach	2.6×10^{-2}	3.2×10^{-4}	0	1.8	3.8
		Lung	2.0×10^{-2}	3.2×10^{-4}	0	1.4	3.4
	Multiplicative	Stomach	47.7 P_0=0.013	1.1×10^{3}	0	6.2×10^{-1}	1.3
		Lung	19.2 P_0=0.038	2.6×10^{2}	0	6.5×10^{-1}	1.7
Rats/ Females	Linearized multistage	Lung, liver, HP/NT			0	1.15×10^{-1}	1.56×10^{-1}

[a]A LADD of 50 pk/kg bw-day via skin and oral route with estimated 5% absorption is equivalent to an administered dose of 100 pg/kg bw-day when exposure is via ingestion with estimated 50% absorption. All the above estimates are standardized to the administered dose via ingestion.

[b]For the additive model the parameter estimates are in units of kg-day/ng-year. For the multiplicative model the estimates are in units of kg-day/ng.

[c]MLE = maximum likelihood estimate. For the additive model, MLE = 70 x Δ ; for the multiplicative model, MLE = P_0 x Δ .

[d]For humans the upper-limits are based on the asymptotic variances. This produces lower upper-limit values.

on the above analysis is that the upper-limit incremental unit risk estimates based on the animal studies do not overestimate the human risk from 2,3,7,8-TCDD if either the lung or stomach cancer mortality response in humans is bona fide.

B. ZACK AND SUSKIND (1980)

1. Description

In Nitro, West Virginia, on March 8, 1949, excessive temperatures in an autoclave involved in the production of 2,4,5-trichlorophenoxyacetic acid caused a relief valve to open, allowing fumes and residues to escape into the atmosphere and into the interior of the building. A total of 121 white males were identified as having developed chloracne following this incident, and these were included in the subsequent follow-up study, with vital status ascertained through the last day of 1978, nearly 30 years.

There was 100% follow-up of subjects; 89 were still alive and "32 were verified deceased by death certificate." With 46.41 expected deaths, the standardized mortality ratio (SMR) for all causes, 69, was significantly ($p < 0.05$) lower than expected. The only cause of death that displayed normal rates was cancer which had 9 observed and 9.04 expected, SMR=99.6. Of those cancer deaths, five were from lung (2.85 expected, SMR=175), three were from lymphatic or hematopoietic tissue (0.88 expected, SMR=341), and one was a soft tissue sarcoma (STS) (0.15 expected). Only one of the cancer deaths, a lung cancer, was a nonsmoker.

2. Risk Estimates

In order to make any kind of quantitative risk estimation of the potency of 2,3,7,8-TCDD, several assumptions must be made. These are:

(a) Exposure. As discussed above for the Holmesburg study, it is assumed that the LADD necessary to cause chloracne was in the 2 to 1,000 pg/kg-day range.

An estimate in the middle, or 500 pg/kg-day, appears to be a reasonable starting point, as does a value of 150 pg/kg-day, which is close to the geometric mean. The value of 500 pg/kg-day will be chosen as the LADD dose for those who developed chloracne, but the uncertainty of this estimate must be stressed. This dose estimate is 10 times greater than that estimated for the BASF study; the incremental unit risk cancer estimates will be correspondingly lower.

(b) Expected deaths from cancer. Table 14 presents the observed and expected cancer deaths for the 29.8-year latency. However, all the cancer deaths appeared after at least a 10-year latency, and it seems reasonable that 10 years is an appropriate latent period for any 2,3,7,8-TCDD-related cancer to express itself. Therefore, the expected deaths presented by Zack and Suskind must be adjusted by subtracting the first 10 years' experience. An examination of vital statistics rates for lung cancer and STS suggests that for lung cancer deaths approximately 20% of a 30-year death experience happens in the first 10 years; for STS the figure is approximately 30%. Based on these adjustments, the expected deaths for this exposed cohort become 2.3 and 0.10 for lung and STS cancers, respectively.

(c) Person-years at risk. A figure needed for the additive risk model but not the relative risk model is the person-years at risk. Since the first 10 years are considered to be a latent or risk-free period, only the last 19.8 years are counted as person-years at risk. The follow-up was complete and there were 32 deaths. It can be assumed that the average time until death was 20 years from first exposure (for the nine cancer deaths the average time from the accident until death was 22 years). Therefore, the total person-years at risk can be estimated as

$$P\text{-}Y = 19.8 \times 89 + 10 \times 32 = 2,082$$

TABLE 14. FOLLOW-UP OF 121 CHLORACNE CASES AT THE MONSANTO COMPANY (NITRO, WEST VIRGINIA) USED TO DERIVE QUANTITATIVE CANCER RISK ESTIMATES

	Lung cancer		STS	
	Unadjusted	Adjusted	Unadjusted	Adjusted
Deaths				
Observed	5	5	1	1
Expected	2.85	2.3	0.15	0.10
Person-years at risk	--	2082	--	2082
SMRs				
Observed	175	217	667	1000
95% Confidence limits based on Poisson distribution	57-410	70-508	9-3707	13-5560

The results of the analysis using both the additive and multiplicate models are presented in Table 15. Based on the assumptions discussed above, both models yield similar results. For extrapolation based on lung cancer deaths, the MLEs for incremental risks are 9.1×10^{-2} and 4.5×10^{-2} $(mg/kg\text{-}day)^{-1}$ for the additive and multiplicative models, respectively. For extrapolation based on the one STS death, the MLEs are lower than are those for lung, 3.0×10^{-2} and 8.6×10^{-3} for the additive and multiplicative models, respectively. The 95% upper-limit estimates presented in Table 15 are those based on asymptotic normality theory and are, therefore, lower than would be produced by applying the upper confidence limits on the SMRs from Table 14, which are based on the Poisson distribution. Those Poisson upper-limit adjusted SMRs, for example, would yield 95% upper-limit cancer unit risk estimates of 5.2×10^{-2} $(ng/kg\text{-}day)^{-1}$ for STS, and 1.6×10^{-1} for lung cancer deaths for the multiplicative model. These values are significantly greater than those presented in Table 15 [1.4×10^{-2} and 1.1×10^{-1} $(ng/kg\text{-}day)^{-1}$, respectively] and reflect the high degree of variability due to small sample size.

Also presented in Table 15 is the quantitative cancer risk estimate derived from the Kociba feeding study (U.S. EPA, 1985). While the MLEs based on the animal data are slightly higher than those based on the Monsanto data, the differences are no greater than a factor of 2.4 for lung cancer and 13.4 for STS. All the 95% upper-limit estimates based on the human data are within a factor of 2 of the upper-limit estimate based on the rat data.

The conclusion based on the above analysis is similar to that derived from the BASF analysis--the available human cancer data on 2,3,7,8-TCDD do not provide any evidence that the unit risk estimate based on rat data overpredict the human experience. The information on human exposure is just too uncertain to allow for a more definitive statement.

TABLE 15. COMPARISON OF INCREMENTAL CANCER RISK ESTIMATES FOR LIFETIME EXPOSURE TO 2,3,7,8-TCDD BASED ON THE KOCIBA MALE RAT LIVER, LUNG, AND HARD PALATE/NASAL TURBINATES (HP/NT) RESPONSE WITH ESTIMATES BASED ON SARCOMA MORTALITY IN 121 MONSANTO EMPLOYEES (ASSUME LADD = 500 pg/kg bw-day)

Species/ sex	Model used	Cancer type	Parameter estimates[b] Δ	Asymptotic variance of estimates	Lifetime incremental cancer risk per 1 ng/kg-day continuous exposure		
					Lower limit	MLE[c]	95% Upper limit[d]
Humans/ Males	Additive	STS	4.3×10^{-4}	2.3×10^{-7}	0	3.0×10^{-2}	8.5×10^{-2}
		Lung	1.3×10^{-3}	1.2×10^{-6}	0	9.1×10^{-2}	2.2×10^{-1}
	Multiplicative	STS P_0=0.00095	9.0	10.0	0	8.6×10^{-3}	1.4×10^{-2}
		Lung P_0=0.038	1.2	0.94	0	4.5×10^{-2}	1.1×10^{-1}
Rats/ Females	Linearized multistage	Lung, liver, HP/NT			0	1.15×10^{-1}	1.56×10^{-1}

[a]A LADD of 50 pk/kg bw-day via skin and oral route with estimated 5% absorption is equivalent to an administered dose of 100 pg/kg bw-day when exposure is via ingestion with estimated 50% absorption. All the above estimates are standardized to the administered dose via ingestion.

[b]For the additive model the parameter estimates are in units of kg-day/ng-year. For the multiplicative model the estimates are in units of kg-day/ng.

[c]MLE = maximum likelihood estimate. For the additive model, MLE = 70 x Δ ; for the multiplicative model, MLE = P_0x Δ .

[d]For humans the upper-limits are based on the asymptotic variances. This produces lower upper-limit values.

V. DISCUSSION AND SUMMARY

This report has presented the effects on the human cancer risk estimates from 2,3,7,8-TCDD exposure under varying assumptions involved in the animal-to-human extrapolation procedure. It has compared EPA's risk estimates with those of other agencies, both U.S. and foreign, discussed the rationale used in each, and shown the effects of slightly different assumptions on the estimates. In general, the risk extrapolations divide roughly into two groups, those agencies using the linearized multistage family of models for extrapolation and those using an uncertainty factor approach. The agencies using the linearized multistage model all produce RsDs within a factor of 10; similarly, the agencies using the uncertainty factor approach are also with a factor of 10. The two groups, however, are separated by a factor of 16, so that the lowest RsD, that of EPA which used the linearized multistage model, is 1,600 times lower than that of Health and Welfare Canada which used an uncertainty factor of 100.

While EPA's cancer risk estimates were the highest of the agencies presented, other methodologies consistent with the data have yielded still higher estimates. For example, fitting the data with both the Logit and the Weibull models would have produced significantly higher estimates--the Logit by roughly one order of magnitude and the Weibull by 2 orders of magnitude. In addition, even extending the multistage model to the Weibull-in-time model under a time-to-tumor analysis would have increased the upper-limit estimates by as much as a factor of 5. Of even greater uncertainty is the extremely long half-life of 2,3,7,8-TCDD in the human compared to the rat. If half-life is related to species sensitivity as implied by the cross-species extrapolation factor, then recent estimates of human half-life of 6 to 10 years implies that rat-to-human extrapolation estimates should be significantly higher, probably by a factor of 2 to 7.

Finally, a new methodology has been presented that models 2,3,7,8-TCDD as a carcinogen promoter only. The methodology models the promotion (liver cell proliferation) phase of the M-V-K two-stage model as a linear saturable function of 2,3,7,8-TCDD-receptor binding. The risk estimates of induced human liver cancer are 2 orders of magnitude less than those using EPA's current methodology. However, research on this new methodology is ongoing and, at this time, not enough is known about the low-dose estimation properties to make definitive statements.

Which of these is the "correct answer"? Probably "none of the above." What the above analyses show are that all the answers are consistent with the observed data, and all have some credence depending upon the believability of the assumptions used. The most pertinent fact is that 2,3,7,8-TCDD causes liver, tongue, hard palate/nasal turbinates, and lung tumors in rats at doses and conditions to which humans would never be exposed. As such, even with animal bioassays, as well-conducted as were those for 2,3,7,8-TCDD, the information they contain for low-dose extrapolation is very limited. Within a 100-fold decrease from experimental dose levels, the range of estimates predicted by models that fit the data well, rapidly diverge to a "pay your money, take your choice" level of 3 orders of magnitude. Below that, divergence is even more rapid (see Tables 4 and 9). Furthermore, when extrapolation is made from animals to humans, the uncertainty about the effect of the extremely long half-life in humans gives concern about the conservativeness of the upper-limit based on the surface area correction for extrapolation.

Use of human data for risk assessment purposes is also impossible with 2,3,7,8-TCDD. First, the evidence for human carcinogenicity of 2,3,7,8-TCDD alone is judged inadequate (see Appendix B). Second, the studies providing positive evidence for carcinogenicity are of a case-control design; these lack both

a population base and exposure estimates. The few cohort studies available lack sensitivity because of a combination of factors including exceptionally small cohorts, insufficient latency, and in some instances, little evidence of exposure. Only with one study was there even an estimate of 2,3,7,8-TCDD exposure, and that study lacked power to discredit any of the predictions provided by the animal data.

Well, the next question is whether any of these estimates can be considered superior to the others. To answer this, one must first presume that mathematical models can be used for prediction, and then decide on whether to model 2,3,7,8-TCDD as a complete carcinogen or as a promoter only. Modeling as a complete carcinogen, only the one-hit and multistage models have a theoretical backing in carcinogenesis; the other models presented are merely well-known tolerance distribution models. The EPA position is that use of the linearized multistage model for extrapolating upper-limits of incremental risk is both prudent and protective. When both animal and human data have been available for risk estimation, the linearized multistage model's use with animal data has provided estimates comparable with those derived from human data. Furthermore, low-dose supralinearity is rarely seen, so that using the linearized multistage model for extrapolating from experimental levels probably represents a protective level for humans.

When one models 2,3,7,8-TCDD as a promoter only, many additional uncertainties arise. The two most important for modeling are those associated with reversibility and threshold. Classical promoters are known to show reversibility of lesions when the promoter is no longer administered and cleared from the system. Furthermore, large doses are typically required for promotion, indicative of a toxicity effect either leading directly to cell damage and regeneration, or overwhelming the cell's ability to prevent the promoter from

reaching its site of action. In the case of 2,3,7,8-TCDD, the evidence for liver cancer in animals points to a mechanism of promotion via receptor binding, yielding a very strongly bound complex. Even if this complex is broken down, the persistence of 2,3,7,8-TCDD allows it to bind with another receptor. In terms of mathematical modeling, this information translates to a dose-response function for cell proliferation which excludes threshold and reversibility, but otherwise can be defined by Michaelis-Menten type kinetics. When this function is substituted into the promoter form of the M-V-K model, the results yield low-dose estimates of liver cancer for humans which are 2 orders of magnitude lower than the upper-limit estimates provided by the one-hit and linearized multistage models.

While the upper-limit estimates can be considered upper-limits for total human 2,3,7,8-TCDD-induced cancer, the predictions with the promoter form of the M-V-K model require further examination. First, they apply only to human liver cancer, a condition reported only in the cohort exposed to dibenzofuran-contaminated PCBs in Yusho, Japan (Amand et al., 1984). They do not include estimates for STS or non-Hodgkins lymphomas. Second, they are considered "best" estimates compared with the upper-limit estimates calculated from the linearized multistage model. Third, the form of the M-V-K model used predicts carcinogenic response only on the basis of promotion. If a 2,3,7,8-TCDD-induced initiation stage had been incorporated (as is suggested by Holder and Rosenthal, 1987) the low-dose cancer predictions would have been higher.

For these reasons, the estimates provided by the promoter form of the M-V-K model might be considered prudent lower bounds on cancer risk, while those upper-limit estimates provided by EPA's current methodology are to be considered upper bounds. While any number of assumptions could produce either lower or higher risk predictions, the biological facts incorporated into both models

provide what should be considered reasonable working limits. In addition, 2,3,7,8-TCDD's many other toxicities should also be a consideration in setting a lower limit. Any criteria level lower than that provided by the M-V-K model would be at a level where these concerns would prevail. A final caveat remains, however, on the impact of the extremely long half-life of 2,3,7,8-TCDD in man. If 2,3,7,8-TCDD is not sequestered in the fat, but is bioavailable, the quantitative risk estimates would be considerably larger.

VI. REFERENCES

Amand, M.; Yagi, K.; Nakajima H.; Takehora R.; Sakai H.; Umed, G.; Labor K. (1984) Statistical observations about the causes of the death of patients with oil poisoning. Japan Hygiene 39(1): (translation)

Armitage, P.; Doll, R. (1954) The age distribution of cancer and a multistage theory of carcinogenesis. Br. J. Cancer 8:1-12.

Barnes, D. (1987) Holmesburg prison-based assessment. Unpublished notes. March 10, 1987.

California. (1985, April 19) Health effects of 2,3,7,8-TCDD and related compounds (draft). Department of Health Services. Berkeley, California. Unpublished.

Centers for Disease Control (CDC) (1980) Serum dioxin in Vietnam-era veterans. Preliminary report. Morbidity and Mortality Weekly Report 36(28):470-475.

Chu, K.C.; Brown, C.C; Tarone, R.E.; Tan, W.Y. (1987) Differentiating among proposed mechanism for tumor promotion in mouse skin with the use of the multievent model for cancer. J. Natl. Cancer Inst. 79(4)789-796.

Crump, K.S. (1983) How to handle animal survival data in quantitative risk assessment: a discussion paper. Prepared for Research Triangle Institute, EPA Contract No. 68-01-6826, Delivery Order 28.

Crump, K.S. (1988) A critical evaluation of a dose-response assessment for TCDD. Food. Chem. Toxicol. In press.

Crump, K.S.; Watson, W.W. (1979) GLOBAL 79: A fortran program to extrapolate dichotomous animal carcinogenicity data to low dose. Prepared for the National Institute of Environmental Health Sciences, Contract No. I-ES-2123.

Crump, K.S.; Guess, H.A.; Deal, L.L. (1977) Confidence intervals and test of hypothesis concerning dose-response relations inferred from animal carcinogenicity data. Biometrics 33:437-451.

Cook, B.T.; Page, N.P. (1986, February 3) Comparison of carcinogenicity risk assessment of 2,3,7,8-TCDD (draft). EPA Contract No. 68-02-4131, Work Order No. 3.

Crockett, P.W.; Crump, K.S. (1986, February) Methods for assessment of non-cancer health risks. Prepared by K.S. Crump and Co. for the Electric Power Research Institute, Contract No. RP 1826-17.

Environmental Canada. (1984) Chlorophenols and their impurities in the Canadian environment. 1983 Supplement. Economic and technical review report. EPS3-EP-84-3. Environmental Protection Programs, Directorate, Canada.

Food and Drug Administration (FDA). (1983, June 30) Statement by S.A. Miller, Director, Bureau of Foods, FDA, before the Subcommittee on Natural Resources, Agriculture Research and Environment, U.S. House of Representatives.

Germany. (1984) Report on dioxins. Update to Nov. 1984. Federal Environmental Agency.

Howe, R.B.; Crump, K.S. (1982) WEIBULL82: a FORTRAN program for low-dose extrapolation using time to tumor data. K.S. Crump and Company, Inc., Ruston, Louisiana.

Holder, J.M.; Rosenthal, S. (1987, February 13) Issue paper concerning the mechanism of 2,3,7,8-TCDD carcinogenicity. Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC. Unpublished draft report.

Kimbrough, R.D.; Falk, H.; Stehr, P.; Fries, G. (1984) Health implications of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) contamination of residential soil. J. Toxicol. Environ. Health 14:47-93.

Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; et al. (1977) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Submitted to the U.S. Environmental Protection Agency. Unpublished.

Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; et al. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46(2):279-303.

Longstreth, J.D.; Hushon, J.M. (1983) Risk assessment for 2,3,7,8-TCDD. In: Tucker, R.E.; Young, A.L.; Gray, A.P., eds. Human and environmental risk of chlorinated dioxins and related compounds. New York, NY: Plenum, pp. 639-664.

Moolgavkar, S.H.; Venzon, D.J. (1979) Two-event models for carcinogenesis. Incidence curves for childhood ad adult tumors. Math Biosci. 47:55-77.

Moolgavkar S.H.; Knudson, A.G. (1981) Mutation and cancer: a model for human carcinogenesis. J. Natl. Cancer Inst. 66:1073-1052.

National Academy of Sciences (NAS). (1977) Drinking water and health. Safe Drinking Water Committee, National Academy of Sciences. Washington, DC.

National Research Council of Canada (NRCC). (1981) Polychlorinated dibenzo-p-dioxins: criteria for their effects on man and his environment. Publ. No. 18474, ISSN 0316-0114. NRCC/CNRC Associate Committee on Scientific Criteria for Environmental Quality, Ottawa, Canada. 251 p.

National Toxicology Program (NTP). (1982) Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Osborne-Mendel rats and B6C3F1 mice. Technical Report No. 209. Research Triangle Park, NC.

Ontario, Canada, Ministry of the Environment. (1985) Scientific criteria document for standard development. No. 4-84. PCDDs and PCDFs.

Paustenbauch, D.J.; Shu, H.P.; Murray, F.J. (1986) A critical examination of assumptions used in risk assessment of dioxin contaminated soil. Regul. Toxicol. Pharmacol. 6:284-307.

Portier, C.; Heel, D. (1983) Low-dose-rate extrapolation using the multistage model. Biometrics 39:897-906.

Rappe C.; Aldersson, R.; Bergquist, P. (1987, January) Sources and relative importance of PCDD and PCDF emissions. Presented at the ISWA/WHO-EURO/DAKOFA Specialized Seminar on Emissions of Trace Organics from Municipal Solid Waste Incinerators, Copenhagen, Denmark.

Rose, J.Q.; Ramsey, J.C.; Wentzlin, T.H.; Hummel, R.A.; Gehring, P.J. (1976) The fate of 2,3,7,8-TCDD following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36:209-226.

Rowe, V.K. (1980) Direct testimony at U.S. EPA's cancellation hearings of 2,4,5-T. Exhibit 865. FIFRA Docket Nos. 415 et al.

Sielken, R.L. (1987) Quantitative cancer risk assessments for TCDD. Food Chem. Toxicol. 25(3):257-267.

Sielken, R.L.; Carlborg, F.W.; Paustenbach, D.J.; Shu, H.P.; Murray, F.J. (1986) Alternative approaches to mathematically analyzing the bioassay data for 2,3,7,8-TCDD (Abstract 1133.) Presented at the 25th Annual Meeting of the Society of Toxicology, New Orleans, LA.

Squire, R.A. (1980) Pathologic evaluations of selected tissues from the Dow Chemical TCDD and 2,4,5-T rat studies. Prepared for the Carcinogen Assessment Group, U.S. Environmental Protection Agency, Contract No. 68-01-5092.

Switzerland. (1982) Environmental pollution due to dimins and furans from communal rubbish incineration plants. Federal Office of Environmental Protection, Bern.

Thiess, A.M.; Frentzel-Beyme, R.; Link, R. (1982) Mortality study of persons exposed to dioxin in a trichlorophenol-process accident that occurred in the BASF on November 17, 1983. Am. J. Ind. Med. 3:179-189.

Tschirley, F. (1986) Dioxin. Scientific American 25(2):29-35.

U.S. Environmental Protection Agency (EPA). (1980) Risk assessment on (2,4,5-trichlorophenoxy)acetic acid [2,4,5-T], (2,4,5-trichlorophenoxy) propionic acid, and 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]. Office of Health and Environmental Assessment, Washington, DC. EPA 600/6-81-003. NTIS PB81-234825.

U.S. Environmental Protection Agency. (1985) Health assessment document for polychlorinated dibenzo-p-dioxins. Office of Health and Environmental Assessment, Washington, DC. EPA/600/8-84/014F. NTIS PB86-122546/AS.

U.S. Environmental Protection Agency. (1986) Guidelines for carcinogen risk assessment. Federal Register 51(185)33992-34003.

U.S. Environmental Protection Agency. (1987) Chapter 6. Epidemiology: recent Kodak study. In: Technical analysis of new methods and data regarding dichloromethane hazard assessments. Office of Health and Environmental Assessment, Washington, DC. External Review Draft. EPA/600/8-87/029A.

U.S. Environmental Protection Agency. (1988) Estimating exposures to 2,3,7,8-TCDD. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington, DC. External Review Draft. EPA/600/6-88/005A.

Weil E.S. (1972) Statistics vs. safety factors and scientific judgement in evaluation of safety for man. Toxicol. Appl. Pharmacol. 21:254-463.

Zack J.; Suskind, R. (1980) The mortality experience of workers exposed to TCDD in a trichlorophenol process accident. J. Occup. Med. 22(1):11-14.

APPENDIX

QUANTITATIVE DOSE-RESPONSE MODEL FOR THE TUMOR PROMOTING ACTIVITY OF TCDD

Prepared by:
Todd W. Thorslund, Sc.D.

ICF-Clement Associates
1850 K Street, N.W., Suite 450
Washington, D.C. 20006

For: The Carcinogen Assessment Group
(Dr. Steven Bayard Project Manager)
Research and Development
U.S. Environmental Protection Agency
EPA Contract No. 68-01-6939

February 17, 1987

1. TECHNICAL SUMMARY

A method is developed in this report for the estimation of cancer risk associated with exposures to TCDD under the assumption that TCDD acts exclusively as a tumor promoter. It is recognized that TCDD may have other potential effects on the mechanisms of carcinogenesis; however, evidence suggests that stimulation of cell growth by direct or indirect mechanisms could play an important role in TCDD's carcinogenic effect.

TCDD's ability to affect cell proliferation can be described using a mathematical dose-response model. A very explicit definition of promotional activity can be used within the context of the Moolgavkar-Knudson-Venzon two-stage model to accomplish this. It is assumed that a promoter can act by increasing the net growth rate of preneoplastic, initiated stem cells and has the ability to increase the growth rate to a fixed upper bound. The difference between this upper bound and the background growth rate is the maximum increase possible. The mathematical function that defines the fractional part of the change in the maximum increases in the growth rate due to a given exposure level of an agent is the critical element in the derivation of a dose-response relationship. Information that can be used to elucidate the form of the critical function that defines the exposure-dependent cell growth rate comes from the three sources: (1) theoretical biological arguments, (2) the shape of tumor dose-response relationships from TCDD carcinogenesis bioassays, and (3) studies of

TCDD-induced cell proliferation rates as direct or indirect measures of cell growth in vivo or in vitro.

In this report, two separate parametric models describing the dose-dependent changes in cell growth are postulated. Each model is consistent with a series of studies on the mechanisms of action of TCDD. The first growth rate model assumes that the changes follow first order kinetics. This assumption results in a negative exponential model that contains only one parameter requiring estimation. This model is fitted to the most extensive data set available (liver tumors in female Sprague-Dawley rats) in order to estimate the three unknown parameters in the tumor dose-response model. The validity of the resulting model is tested in four different ways: (1) its goodness-of-fit to the tumor data, (2) its consistency with TCDD-induced proliferation data, (3) its ability to predict the TCDD-induced tumor response in other sexes and strains of the same species (rats) using adjustments only for background tumor rates, and (4) its ability to predict the TCDD-induced tumor rates in another species (mice), making adjustments for background rates and estimating a separate growth rate parameter for that species.

A second model is investigated that assumes the rate of change of the TCDD-induced growth rate is proportional to the product of cellular TCDD levels and the number of TCDD receptors. The resulting model for the increased cell growth rate is log-logistic in form. This model has two parameters, the slope and

intercept; only the latter can be estimated with the available tumor dose-response data. In order to use this model, the slope parameter needs to be specified. This is done by specifying slopes that span the range of those determined from other types of biological systems that are log-logistic in form. The intercept associated with a slope is then estimated using the bioassay data.

Both models are used to extrapolate from observed animal tumor rates to expected human response rates. The age-specific human death rates due to liver cancers are used to estimate two of the parameters in the human model. The use of human data for this purpose is mandated by two factors. Under the assumed model, the agent acts on initiated, preneoplastic cells to increase their number. As a result, the increase in the tumor rate should be proportional to the background number of preneoplastic cells, which in turn is proportional to the background tumor rate. This theoretical prediction is confirmed by the strong dependence of the sensitivity of the tumor response to the background tumor rates observed in five separate animal bioassays. The usual assumption of the equivalent dose between species being proportional to the cube root of the ratio of the species weight is also employed in the development of the human dose response model. Using the developed models it is demonstrated that low-dose linearity can result from either form of the models under certain circumstances. In contrast, is is also shown that the log-logistic model with a slope equal to 3 gives prediction of risk that decrease 3 orders of magnitude for each order of magnitude of reduction of exposure.

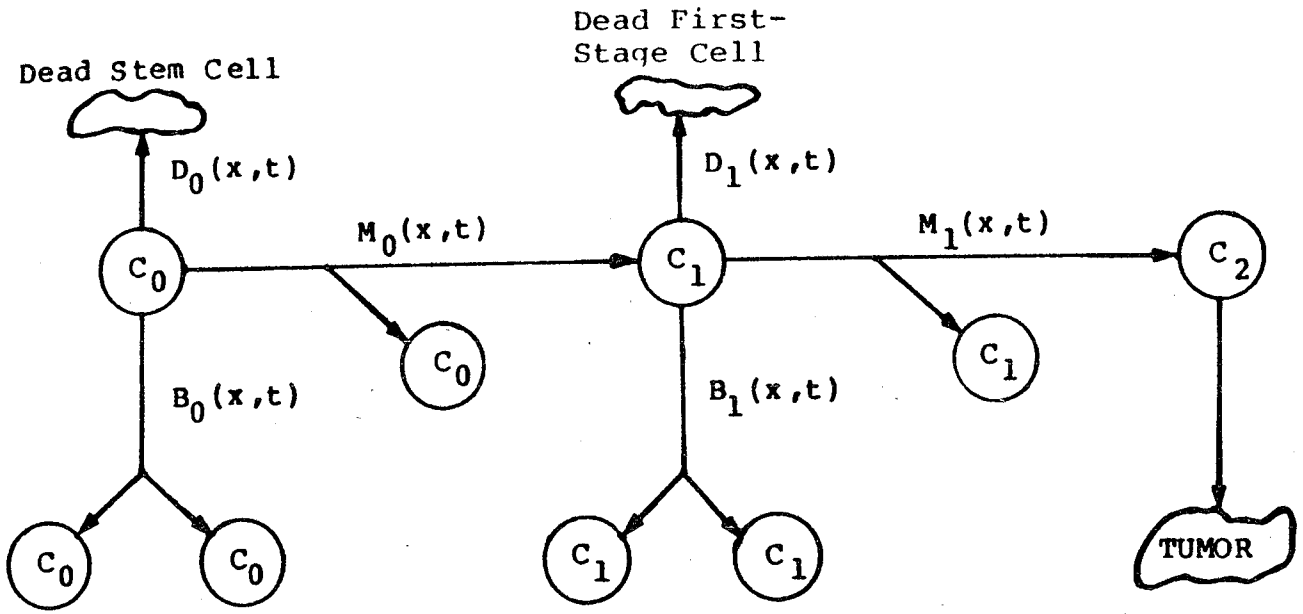
While both forms of the M-V-K model fit the observed data quite well, and both can be justified on theoretical and some experimental ground, their use for low-dose extrapolation leads to a wide variation in risk estimates. For a lifetime daily dose of 0.1 ng/kg-day, or one order of magnitude below the animal experimental level, the estimates vary by a factor of more than 10,000.

Although the negative exponential form of the model results in a linear low-dose response relationship, the risk estimates derived from it are two orders of magnitude below the upper bound values obtained by EPA when TCDD was modeled as a complete carcinogen. This difference is primarily due to the low human background liver cancer rates as compared to those of the female rat. Thus, treating TCDD as a promoter could have a strong impact on any regulatory decision. However, the model is based upon the assumption that the site of cancer induction in humans would also be in the liver. An analysis of tissue dose distribution and cell turnover rates and receptor protein levels for other organs or tissues should be conducted before the equivalence of target site between humans and rodents is accepted unequivocally.

2. MATHEMATICAL DOSE RESPONSE MODEL FOR PROMOTERS

A mathematical model has been developed by Moolgavkar, Venzon and Knudson (1979, 1981) that is based on a two-stage model for carcinogenesis. This model is depicted in Figure 1.

FIGURE I GENERAL BIOLOGICAL STRUCTURE UNDERLYING EXPOSURE- AND TIME-DEPENDENT M-V-K MODEL



KEY:

C_0 is a normal, susceptible stem cell.
C_1 is a preneoplastic, first-stage cell, which can proliferate into a premalignant clone.
C_2 is a cancerous cell that will eventually develop into a detectable tumor.
$D_0(x,t)$, $B_0(x,t)$, and $M_0(x,t)$ are the exposure- and time-dependent death, birth, and transition or mutation rates for the normal stem cell.
$D_1(x,t)$, $B_1(x,t)$, and $M_1(x,t)$ are the exposure- and time-dependent death, birth, and transition or mutation rates for the first-stage cell.
x is the exposure level, which is assumed to be constant over time.
t is the age of the subject.

According to the model, the population of cells at risk is proliferating cells, often referred to as stem cells. Stem cells are those from which most other cells in an organ arise; once a cell has differentiated and left the pool of proliferating cells it is no longer susceptible to heritable alterations of its DNA. A normal, susceptible stem cell may do one of several things. It may divided into two daughter stem cells, terminally differentiate, die, or undergo mutation at a critical site that results in formation of a preneoplastic or intermediate cell. A preneoplastic cell has undergone one of the changes necessary to become a cancer cell but is not yet cancerous. The cancerous cell will, after a sufficient length of time, divide into enough cells that it becomes a detectable tumor. All of these processes can be described mathematically by postulating specific rates for the cell changes. Moolgavkar and Knudson (1981) showed that to a close approximation, the age-specific tumor rate at age t for their two-stage model may be expressed as follows:

$$I(t) = M_0 M_1 \int_o^t C_0(v) \left\{ \exp\left[(B-D)(t-v) \right] \right\} dv \qquad (1)$$

where

$I(t)$	=	age-specific cancer incidence at age t;
M_0	=	transition rate from stem to preneoplastic cell;
M_1	=	transition rate from preneoplastic to cancerous cell;
$C_0(v)$	=	number of susceptible stem cells at age v;
B	=	birth rate or rate of cell proliferation of preneoplastic cells; and
D	=	death rate of preneoplastic cells.

Essentially, the equation describes the progression from a normal stem cell to a cancerous cell under the assumptions of the two-stage model. This model is a combination of deterministic and stochastic components. The numbers of preneoplastic and fully malignant cells at any time are assumed to be random variables that are dependent upon these event rates, while the number of normal cells at risk of transformation at time t, denoted by $C_0(t)$, is assumed to be deterministic and known.

The biological processes described by the equation 1 can be affected by the level of exposure to carcinogenic agents, and are more likely to occur as the length of exposure increases; as a result, they are exposure and time dependent. Thorslund et al., (1987) have described an exposure- and time-dependent version of the model. In the context of biological mechanisms of carcinogenesis, the model can be used to predict the risk of agents that exert their effects in a number of different ways. Mutation-inducing initiating agents could affect the transition rates between cell stages (M_0 or M_1), while promoting agents may increase the proliferation rate of preneoplastic cells (increasing B without affecting D). Cocarcinogens may increase the proliferation rate of normal stem cells (C_0), thereby increasing the size of the target for initiating agents. Inhibitors could remove cells from the populations of susceptible stem or preneoplastic cells through toxicity (e.g., increasing D without affecting B) or by inducing differentiation.

For the purposes of the model, a mechanism of action for TCDD has been postulated that is similar to that of a promoting agent. It has been assumed that TCDD exerts its carcinogenic effect by increasing the preneoplastic cell birth rate. The difference between the birth and death rates is the net preneoplastic cell growth rate and is dose-dependent. This rate is denoted as:

$$G(x) = B + B_o(x) - D \qquad (2)$$

where $G(x)$ = dose-dependent preneoplastic cell growth rate

$B_o(x)$ = the increase in the birth rate due to TCDD

B, D are as defined in equation (1)

Upon maturity, the number of stem cells in the liver may be viewed as relatively constant. Under this assumption, the term $C_o(v)$ may be taken to be equal to a non-time-dependent constant, C_o. Substituting these definitions for $G(x)$ and $C_o(v)$ into equation (1) and integrating yields:

$$I(x,t) = M_0 M_1 C_o [\exp G(x)t - 1]/G(x) \qquad (3)$$

which is the age-specific tumor rate expressed in a dose and time dependent form. The probability of a tumor by time t in the absence of competing mortality is obtained from the well-known relationship:

$$P(x,t) = 1 - \exp - \int_o^t I(x,v)\,dv \qquad (4)$$

Substituting equation (3) into equation (4) and integrating yields the expression:

$$P(x,t) = 1-\exp-\left\{M[\exp G(x)t-1-G(x)t]/G(x)^2\right\} \qquad (5)$$

where $M = M_o M_1 C_o$ is a composite parameter that is proportional to the background tumor rate. The growth rate has a normal value $G(o) = B-D$ in the absence of exposure and has a finite upper bound $G(\infty)$ that is determined by how rapidly a cell can go through its normal proliferative cycle. Using this notation, the exposure-dependent growth rate of preneoplastic cells may be expressed as:

$$G(x) = G(o)+[G(\infty)-G(o)]R(x) \qquad (6)$$

where $R(x)$ is the fractional amount of the maximal increase in the growth rate that can be induced by exposure at level x. The function $R(x)$ is dependent upon the specific mechanism by which TCDD induces cell proliferation. A specific form for $R(x)$ can be derived for each hypothesized mechanism of action. The next section discusses the factors that need to be considered in the selection of the $R(x)$ to be used in equation (6).

3. MECHANISMS OF THE ACTION

Carcinogenesis is a multistep process which displays at least two distinct steps. External agents (carcinogens) can act to augment the process at each of these steps (Weinstein, 1981). For simplicity, carcinogens are often divided into two classes: "initiators" (those that act at the first stage of cancer development) and "promoters" (those that act at later stages and hence

"promote" the action of initiators) (Weisburger and Williams, 1983; Weinstein, 1984). However, recent studies have shown that both initiation and promotion are more complex phenomena than is reflected in this dichotomous classification (Becker, 1984; Upton, et al., 1985; Gallagher, 1986). Promotion frequently involves the action of promoters on cell membranes, including binding to protein kinases, inhibition of intercellular communication, and other effects on membrane structure and function, but also may involve genetic changes, including methylation of DNA, modulation of expression of genes involved in cell differentiation, activation of oncogenes, and chromosome damage (Shank, 1984; Yamasaki and Weinstein, 1985; Weinstein, 1984; Jones, 1986).

It is unlikely that TCDD is a typical initiator. There is little evidence that it causes point mutations in bacteria (e.g. in the Ames test) but it has some genotoxic potential, as reflected by a clear positive result in the mouse lymphoma assay (Rogers, et al., 1982). TCDD has little propensity for covalent binding to DNA (Poland and Glover, 1979); however, it is known that after binding of TCDD to a cytosol "receptor" protein, the TCDD-receptor complex is translocated to the nucleus of the cell where it interacts with different sites on DNA and can affect various functions. These include regulating transcription of the cytochrome P-448 gene (Israel and Whitlock, 1984; Whitlock, et al., 1984; Jones, et al., 1985); regulating the levels of other enzymes such as ornithine decarboxylase, DT-diaphorase, and UDP-glucuronyl transferase; and affecting the regulation of cell proliferation and differentiation by interfering with the ability

of other sites on DNA to bind receptor complexes such as those of epidermal growth factor and glucocorticoids (Poland and Knutson 1982).

The latter two functions may be those by which TCDD affects tumor promotion. Agents that increase the number of proliferating cells by increasing cell proliferation rates or decreasing the number of cells that terminally differentiate could increase the likelihood of mutational events by tumor initiators or permit clonal expansion of initiated cells. The mechanism of enhanced cell proliferation may involve the interaction of a single TCDD-receptor protein complex with a single site on DNA (such as that of a regulator gene), which would be consistent with a fractional change in a growth rate function of the form $R(x) = 1-\exp-Vx$. Alternatively, TCDD's action may require the formation of multiple complexes with roles at multiple sites on DNA, which would suggest a log-logistic relationship for

$$R(x) = [1+\exp-(I+S \ln x)]^{-1}.$$

Studies performed to date evaluating the ability of TCDD to cause hepatic cell proliferation are inconclusive. Conaway and Matsumura (1975), for example, found an increase in hepatic nuclear ^{3}H-thymidine activity as compared to controls of 56% or 94%, depending on the method of preparation, ten days after administration of a single oral dose of 5 ug/kg TCDD to male Sprague-Dawley rats. Dickens et al. (1981) saw an increase of 94% in ^{3}H-thymidine activity associated with hepatic DNA five days after

a similar dose although the difference was not statistically significant due to the small number of animals used. These authors also noted significant increases in liver net weight and relative liver weight compared to body weight in treated animals. In a similar experiment, however, Christian and Peterson (1983) saw no such increases. Further studies are clearly needed to resolve the discrepancies along with studies of the dose-response and time-course relationships of any increases observed.

In the absence of detailed, reliable data on the dose-related effects of TCDD on cell growth rates, we shall assume two functional forms for R(x):

(1) $R(x) = 1-\exp-Vx$ which would be appropriate if the proliferation effect was due to the interaction of a single TCDD-receptor protein complex with a single site on DNA, or

(2) $R(x) = [1+\exp-(I+S \ln x)]^{-1}$ which would be appropriate if the interaction of multiple TCDD-protein receptor complexes at multiple sites were required for stimulation of the growth rate.

4. RATIONALE FOR THE SELECTION OF THE DATA USED TO DEVELOP TUMOR DOSE RESPONSE MODEL

A number of decisions regarding the most appropriate study, tumor end point, time frame, pathological diagnosis, and exposure parameter must be made in order to obtain the specific data set that is used to estimate the parameters in the postulated dose response models. The rationale for those decisions is discussed in this section.

4.1 Selection of Animal Tumor Data

As discussed in the Health Effects Assessment Document for Polychlorinated Dibenzo-p-Dioxins (USEPA, 1985), several chronic animal studies of TCDD have demonstrated enhanced liver tumor rates. In its extrapolation from animal data to human cancer risk estimates, EPA used the hepatic tumor rates in female Sprague-Dawley rats from the Dow 2-year feeding bioassay (Kociba et al., 1978). These data are discussed in some detail in both the HAD and in the issue paper on the quantitative implication of the use of different models. Every U.S. and foreign agency except one (California) has used the DOW study as its pertinent cancer study for extrapolating to humans. In this report, only the liver response will be modeled. While the female rats in this study also developed rare tumors of the hard palate/nasal turbinates as well as infrequent lung tumors, neither of these tumor types has been shown to be the result of promotion. On the other hand, TCDD has been shown to be a potent promotor in the rat liver (Pitot et al., 1980).

In its extrapolation procedure for TCDD, EPA uses the pathology analysis results of two independent pathologists, Dr. R. Kociba and Dr. R. Squire. Even though Squire's analysis included more liver tumors in the control, low- and mid-dose groups, the estimates of incremental increase in cancer risk differed by less than 10%. This report will use the Squire pathology analysis of the data. Although the higher background

rates based on the Squire analysis lead to higher incremental cancer risks for animals under a promotor model, the human background rates are substituted when the results are extrapolated to humans. The final estimates derived for humans should be similar, therefore, since they depend only on differences in response between the animal dose groups, which were about the same according to both pathologists.

In an attempt to adjust for high early non-tumor related mortality in the high dose group, EPA eliminated from consideration all animals dying prior to the 13th month, when the first liver tumor was observed. This censored data set also will be used here.

4.2 Selection of Exposure Variable

Rose et al. (1976) applied a simple first-order (i.e., one compartment open) model to estimate the steady-state concentrations of TCDD in the liver of female Sprague-Dawley Spartan rats. Based upon their analysis, it was concluded that the administered dose following oral exposure was proportional to the target organ dose (i.e., steady-state liver dose) in the oral dose range of 0.01-1.0 ug/kg/day TCDD; however, actual steady-state doses were not measured. Kociba et al. (1978) measured the levels of TCDD at the end of a two-year carcinogenesis bioassay in five rats from each exposure group receiving 0.001, 0.001 or 0.1 ug/kg/day. Levels of 0.54, 5.1, or 24 parts per billion, respectively, were observed. Portier et al. (1984) used these exposure levels to

fit dose-response models to the tumor data. To extrapolate exposure levels from those received in the bioassay to the lower levels encountered environmentally, the relationship between administered and target organ dose was assumed to be linear from 0.01 ug/kg/day to zero. This approach has several problems. The results are based on small sample sizes with considerable variability between measurements. Potential biases may also have been introduced by obtaining observations only at terminal sacrifice and by having high tumor rates and liver weight increases at the highest dose level, which distort the levels of TCDD per cell when measured as proportional to liver weight. As a result of these problems, the average liver exposure level data will not be used to derive dose-response models. Nevertheless, since the value of the highest dose used in the bioassay is the only one that would be altered by using these data, it can be shown that they have virtually no influence on the estimate that results from the models employed in the subsequent analysis. Furthermore, the use of administered doses is consistent with EPA's previous approaches so that direct comparisons are more meaningful.

5. PARAMETER ESTIMATION

The two models that will be used to predict risk may be expressed as:

$$P(x,t) = 1-\exp-M\left\{[\exp G(x)t-1-G(x)t]/G(x)^2\right\} \qquad (8)$$

$$G(x) = G(o)+[G(\infty)-G(o)]R(x)$$

$$R(x) = \begin{cases} 1-\exp-Vx & \text{case 1} \\ [1+\exp-(I+S\ \ln\ x)]^{-1} & \text{case 2} \end{cases}$$

The number of parameters to be estimated is 4 (i.e., M, G(o), $G(\infty)$, V) for the negative exponential preneoplastic cell growth model (case 1), and 5 (i.e., M, G(o), $G(\infty)$, I, S) for the log-logistic preneoplastic cell growth model (case 2). This section explains how the parameters were estimated and presents the results obtained for the most informative dose response relationship.

As discussed in the previous section, the most reliable and biologically meaningful data set that can be used to obtain a dose response relationship for TCDD is liver tumors in female rats surviving one year in the DOW study using the Squire pathology analysis. This data set is shown in Table 1.

Of the four or five unknown parameters in the models, M and G(o) do not depend on exposure. The parameter M is proportional to the product of the background cell transition rates and G(o) is proportional to the preneoplastic cell growth rates. If time-to-tumor data were available, these parameters could be estimated from control data. When x=o the exposure-time model has the form:

$$P(o,t) = 1-\exp[-MG(t)] \qquad (9)$$

where $G(t) = [\exp(G(o)t)-1-G(o)t]/G^2(o)$.

In the absence of reliable time-to-tumor data, G(o) may be specified based upon knowledge of cell turnover rates or time-

dependent tumor occurrence. The approach taken here was to find a range for G(o) that is consistent with the age-specific tumor rate increasing from the 3rd to the 5th power of age. This range was found by Cook et al. (1969) to be consistent with the tumor registry data for most tumor sites in eleven different populations. Using the multistage model, the probability of a tumor occurrence by time t may be expressed as

$$P(o,t) = 1-\exp-At^k \qquad \text{where } 4 \leq k \leq 6 \qquad (10)$$

An estimate of A can be obtained by fixing k and and substituting a background rate estimate at a fixed time in equation (10), yielding

$$-\ln[1-P(o,t)]/t^k = A \qquad (11)$$

The background rate is obtained from the vehicle control, which results in an estimate of

$$P(o,t) = 16/85 = 0.1882$$

Substituting t=104, P(o,t)=0.1882, and k=4 or 6 into equation (11) gives the results $A=1.78\times10^{-9}$ when k=4 and $A=1.65\times10^{-13}$ when k=6. To estimate G(o), we transform equation (9) to the form

$$\ln\{-\ln[1-P(o,t)]\} = \ln(M/G(o)^2)+\ln[\exp G(o)t-1-G(o)t] \qquad (12)$$

which for $G(o)t \geq 3$ is approximated closely by the simple relationship

$$\ln\left\{-\ln[1-P(o,t)]\right\} = \ln(M/G(o)^2)+G(o)t \qquad (13)$$

This equation is then equated to numerical values obtained from equation (10) at t=52,104, which gives two linear equations and two unknowns for each k that can be used to solve for $\ln(M/G(o)^2)$ and $G(o)$. These values are used in turn to estimate M. Following this approach the values shown in Table 2 were obtained. The purpose of this manipulation is simply to obtain a time-to-tumor relationship for the M-K-V model that corresponds to that which has been previously observed often in terms of the multistage model. Taking the range of the parameters is done in order to investigate the sensitivity of the assumed parameters in the final risk estimates. The observed tumor data are not used to estimate $G(o)$ so that a valid goodness-of-fit test can be obtained from the four data points, which is an added advantage of the approach. The remaining two parameters, $G(\infty)$ and V, are obtained by equating the parametric form of the model to the observed rates at the two highest doses and solving the resulting simultaneous non-linear equations for two unknowns. This is done for both values of k but since the resulting models give virtually identical final results, only the values for k=4 are shown in Table 3, along with the corresponding expected values under the model.

The log-logistic cell growth model is also fitted to the data. This is done by assuming the slope is equal to 1,2, or 3

common values in many biological systems and estimating the intercept corresponding to each assumed slope value. As can be seen in Tables 1 and 3, values of the parameters that provide the best log-logistic fit to the data are S=3 and I=14.5. The results of this analysis are displayed in Table 3. The estimates for $G(\infty)$ and I were obtained from the tumor response data using the same methodology as was done for the negative exponential model.

In the next section the validity of the fitted models is tested in a variety of different ways.

6. EVIDENCE FOR THE VALIDITY OF THE OBTAINED DOSE RESPONSE MODELS

The validity of the dose response models that were obtained in the previous section can be evaluated in a number of ways. Since the models are a subset of the M-V-K model, which has been shown to have a remarkable ability to describe a variety of different carcinogenic phenomena and age-specific cancer rates in humans, a degree of acceptance for their application to TCDD should be accorded on purely theoretical grounds.

In addition, the predictions of the model can be evaluated using (1) the goodness-of-fit of the model to the tumor data from which it was derived, (2) its consistency with TCDD-induced proliferation data from separate experiments, (3) its ability to predict the TCDD-induced tumor responses in other sexes and

strains of the same species (rats) using adjustments only for background tumor rates, (4) its ability to predict the TCDD-induced tumor rates in another species (mice), making adjustments for background rates and estimating a separate growth rate parameter for that species, and (5) its consistency with the prediction that the slope of the linear relationship between the log of the age-specific tumor rate regressed against age will be a monotonically increasing function of exposure.

In this section, the models are evaluated using the first four of these criteria. The consistency of the dose-dependent slope (i.e., criterion (5)) was not attempted due to time and resource constraints.

6.1 Goodness-of-Fit of Models to DOW Female Rat Data

The goodness-of-fits of the hypothesized models to the data set from which they were derived are shown in Table 1. As is indicated by the X^2 values, both models fit the data adequately. A better fit could have been obtained by letting the background rate parameter deviate from the observed background; however, considerable attention has been paid to the fact that the low-dose tumor response is lower than the control tumor response, which has been suggested to imply that some type of unspecified compensatory low-dose mechanism is operating (see Sielken, 1987). The goodness-of-fit test for the model fitted using only three data points has a more specific meaning and greater power than

using all the exposure levels to obtain parameter estimates. The null hypothesis in this case is that the low-dose information is consistent with our hypothesized tumor dose response model. As indicated previously, there is no evidence that is inconsistent with this hypothesis, which is a stronger criterion than a general goodness-of-fit model. The better fit of the log-logistic model compared to the negative exponential model should not be interpreted as suggesting that the former model is more valid, since both models fit the data adequately. Equivalently, the slight improvement in fit due to increasing the slope in the logistic model should not be viewed as strong evidence for a large slope. We note in Table 3 that the log-logistic model fits the data adequately for slope values from 1 to 3, even though, as is indicated by the estimated rat cancer risks, the implications for low dose extrapolation are very different. To illustrate the type of results one might obtain with a log-logistic model, we use the case with a slope of 3 in the final evaluation in addition to the negative-exponential model.

6.2 Consistency with Cell Growth Rate Data

The form of R(x) (i.e., dose-dependent change in growth rate) is obtained from theoretical considerations and the shape of the liver tumor dose-response model. Ideally, the growth rates of hepatocellular adenomas would be used to estimate the parameters in R(x). The hepatocellular adenomas induced by TCDD are thought to be a preneoplastic stage of hepatocellular

carcinomas. A more practical but less direct alternative would be to measure the TCDD-induced growth or turnover rates in normal liver cells in the same species for which TCDD-induced tumor dose response information exists. One measure that can be used in this regard is the H^3 thymidine levels incorporated in liver cell nuclei after TCDD exposure. As cited by EPA (1985), page 8-20, it was shown that the ^3H-thymidine activity at control and a 5 ug/kg TCDD exposure levels were 29 and 45 cpm/mg liver, respectively. We assume that the growth rate is proportional to this index and that the maximum growth rate is obtained at the 5 ug/kg exposure level. If multiple exposures had been used, it might have been possible to define the shape of R(x), as is indicated in Figure 1. However, it is possible to obtain an estimate of G( ) from the limited data available. The ratio of the maximum to minimum growth rates (G( )/G(o)) can be estimated by taking the ratio of the H^3 thymidine counts. Multiplying this ratio (45/29) by the assumed G(o)=0.0533 gives the value G( ) = (45/29)x0.0533=0.0827, which compares closely with the value of 0.0817 obtained from the shape of the bioassay curve.

6.3 Consistency of Model With Other Rat Dose-Response Data

Under the assumed M-V-K model, a promoting agent acts on initiated, preneoplastic cells to increase their number. As a result, the increase in tumor rate should be proportional to the background number of preneoplastic cells, which in turn is proportional to the background tumor rate. The background tumor

rate parameter M should be different, therefore, for strains and sexes that have varying background liver tumor rates. To test the validity of the derived model, all parameters except M were assumed to be known and the resulting model was fitted to the NTP bioassay data for male and female Osborne-Mendel rats. The results of this analysis are shown in Tables 4 and 5 for female and male rats respectively. We note that the resulting model gives an adequate fit to both data sets even though the non-background tumor rates have little weight in the parameter estimation.

6.4 Consistency of Model With Mice Dose-Response Data

Due to biological differences, we would expect that enzyme and receptors levels would vary between species. As a result, in addition to background levels, a parameter for the exposure-induced change in the growth rate V would also have to be estimated when using a different species. For the male mice data in the NTP study, two parameters (M and V) were estimated and the other parameters taken from the previous model. For the female data, only one parameter (M) was estimated while V was obtained from the male data. The results of this analysis are shown in Tables 6 and 7 for male and female mice, respectively. We note that the resulting models also are consistent with the observed data.

7. HUMAN DOSE RESPONSE MODEL

In this final section, human age-specific cancer death rate data and parameters estimated from the animal bioassays are combined to obtain a human dose-response model. The rationale for this approach is that the background tumor rate parameter is the critical factor in determining species sensitivity. This rationale is supported by the results in Table 8, which demonstrate the consistency of risks among species after adjusting for background rates.

The human age-specific liver cancer death rates can be used to obtain estimates of the human parameters M and G(o) under the assumptions that most liver cancers are fatal and that these rates represent actual background rates. Under the model, the age-specific background tumor rates may be expressed as

$$I(t) = M[\exp(G(o)t) - 1]/G(o) \qquad (14)$$

Since we expect $G(o)t \geq 3$ for most of the observed age range, taking the natural log of eq. 14 yields

$$\ln[I(t)] \simeq \ln [M/G(o)] + G(o)t \qquad (15)$$

Values for I(t) are derived from Table 1-25, Section 1, Vol. II, Part A, of the Vital Statistics of the United States 1980 (U.S. HHS, 1985) and the 1980 U.S. Census.

Using these data, a simple linear regression of the form $\ln[I(t)]=A+Bt$ yields a correlation coefficient $r=0.99$ with values $A=-15.6870$ and $B=0.09381(\text{years})^{-1}$ (see table 9). M and G(o) are then estimated by setting the linear regression equation equal to eq.(15). Since A and B are now known, the intercept $A=\ln[M/G(o)]$ and $B=G(o)$. Estimates of the parameters are thus

$$M=B(\exp A)=1.44\times10^{-8} \text{ and } G(o)=.09381 \ (\text{years})^{-1} \qquad (16)$$

The maximum growth rate $G(\infty)$ is assumed to be the same percent change over background as was observed in the DOW female rat data. Thus, for the human model $G(\infty)=0.09381\times(0.0817/0.0533)=0.1438$. Finally, the parameters V and I for humans are obtained from the DOW female rat study by using the usual surface area correction $(70/0.45)^{1/3}=5.38$. The value of the human parameters so obtained are shown in Table 10. The resulting mathematical extrapolation model using these parameters and risks at various low environmental exposure levels are shown in Table 11.

Table 1 - Comparison of Observed and Predicted Liver Tumor Rates in the DOW TCDD Feeding Study Using Female Rats

Exposure ug/Kg/day (X)	Number of Animals Surviving First Year	Number (%) of Animals With Liver Tumors		
		Observed	Predicted	
			1st order Equilibrium	Log-Logistic S=3
0.0	85	16 (19%)	16.0 (19%)	16.0 (19%)
0.001	48	8 (16%)	10.8 (23%)	9.4 (20%)
0.01	48	27 (56%)	27.0 (56%)	27.0 (56%)
0.1	40	33 (82%)	33.0 (82%)	33.0 (82%)
		x^2	0.96	0.16
		d.f.	1	1
		P value	0.32	0.78

Parameters			
Symbols	Estimates		Values Derived From
	1st order	Log-Logistics	
G(o)	0.0533		See text
G(∞)	0.0817		Estimated From Data
M	2.3798×10^{-6}		Estimated From Data
V	109.51		Estimated from Data
I		14.50	Estimated from Data
S		3.00	Assumed

$$P(x,t) = 1-\exp-M\ [\exp G(x)t-1-G(x)t]/G^2(x)\ ,\ t=104$$

$$G(x) = G(o)+[G(\infty)-G(o)]R(x)$$

$$R(x) = 1-\exp-Vx \quad \text{1st order equilibrium (negative exponential)}$$

or

$$R(x) = [1+\exp-(I+S\ln x)]^{-1} \quad \text{Log-Logistic}$$

a Goodness-of-fit assuming that TCDD stimulates cell proliferation using two forms of the promotion model and the Squire pathology analysis, adjusting for early mortality.

Table 2 - Parameter Estimates For The Moolgavkar-Knudson Model That Simulate Range of Multistage Time to Tumor Model for Usual Range of Human Data

Assumed Value of Time Parameter	Resulting Parameter Estimates	
	G	M
4	0.0533	2.15×10^{-6}
6	0.0800	3.03×10^{-7}
1980 U.S. Vital Statistics ICD 155. Liver Cancer Deaths	0.0631[a]	1.44×10^{-8}

a Value adjusted by $.0938(\text{years})^{-1} \times (70 \text{ years}/104 \text{ weeks})$

Table 3 - Effects of Varying Slope Parameter in Log-Logistic Model on Goodness-of-Fit and Low-Dose Risk Estimates Based on DOW Study

Parameter Symbol	Parameter Estimate			
M	2.3798×10^{-6}			
G(o)	0.0533			
G(∞)	0.0817			
I	5.29	9.90	14.50	
S	1	2	3	∞
Augmented Rat Risk at				
$x=1\times10^{-3}$	6.3×10^{-2}	6.6×10^{-3}	6.6×10^{-4}	0
$x=1\times10^{-4}$	6.6×10^{-3}	6.6×10^{-5}	6.6×10^{-7}	0
x^2	2.066	0.245	0.155	0.146
P	0.18	0.62	0.72	0.73

Table 4 - Comparison of Observed and Predicted TCDD-Induced Liver Tumor Rates In the Osborne-Mendel Female Rats From the NCI Gavage Study

Exposure ug/kg/day (X)	Number of Animals Exposed	Number (%) of Animals with Liver Tumors	
		Observed	Predicted
Historical Control	970	21 (2.2)	
0	75	5 (6.7)	2.8 (3.7)
0.0014	49	1 (2.0)	2.4 (4.9)
0.0071	50	3 (6.0)	5.4 (10.8)
0.0714	49	14 (28.6)	13.2 (26.9)
		x^2	3.99
		d.f.	3
		P value	0.28

Values of Parameters (see text)			
Symbols	Biological Interpretation	Estimates	Values drived from
G(o)	background cell growth rate	0.0533	Assumed (A priori)
G(∞)	maximum cell growth rate	0.0817	Dow Study
V	incremental rate change per unit dose	109.51	Dow Study
M	background mutation rate	4.3036×10^{-7}	Estimated From Data

$$P(x) = 1-\exp-M \left\{ [\exp G(x)t-1-G(x)t]/G^2(x) \right\}$$

$$G(x) = G(o) + [G(\infty)-G(o)][1-\exp-Vx]$$

Table 5 - Comparison of Observed and Predicted TCDD-Induced Liver Tumor Rates in Osborne-Mendel Male Rats from the NCI Gavage Study.

Exposure ug/kg/day (X)	Number of Animals Exposed	Number (%) of Animals With Liver Tumors	
		Observed	Predicted
Historical Control	975	9 (0.9)	
0	74	0 (0.0)	0.3 (.4)
0.0014	50	0 (0.0)	0.2 (.5)
0.0071	50	0 (0.0)	0.6 (1.2)
0.0714	50	3 (6.0)	1.6 (3.2)
		x^2	2.37
		d.f.	3
		P value	0.58

Values of Parameters (see text)			
Symbols	Biological Interpretation	Estimates	Values drived from
G(o)	background cell growth rate	0.0533	Assumed (A priori)
G(∞)	maximum cell growth rate	0.0817	Dow Study
V	incremental rate change per unit dose	109.51	Dow Study
M	background mutation rate	4.420×10^{-8}	Estimated From Data

$$P(x) = 1-\exp-M\left\{[\exp G(x)t-1-G(x)t]/G^2(x)\right\}$$

$$G(x) = G(o) + [G(\infty)-G(o)][1-\exp-Vx]$$

Table 6 - Comparison of Observed and Predicted TCDD-Induced Liver Tumor Rates in B6C3F1 Male Mice from the NCI Gavage Study.

Exposure ug/kg/day (X)	Number of Animals Exposed	Number (%) of Animals With Liver Tumors	
		Observed	Predicted
0	73	15 (20.5)	15.00 (20.5)
0.00143	49	12 (24.5)	10.40 (21.2)
0.00714	49	13 (26.5)	11.77 (24.0)
0.07143	50	27 (54.0)	27.77 (55.5)
		x^2	0.53
		d.f.	2
		P value	0.77

Values of Parameters (see text)			
Symbols	Biological Interpretation	Estimates	Values drived from
G(o)	background cell growth rate	0.0533	Assumed (A priori)
G(∞)	maximum cell growth rate	0.0817	Dow Study
V	incremental rate change per unit dose	13.19	Estimated From Data
M	background mutation rate	2.6248×10^{-7}	Estimated From Data

$$P(x) = 1-\exp-M \left\{ [\exp G(x)t-1-G(x)t]/G^2(x) \right\}$$

$$G(x) = G(o) + [G(\infty)-G(o)][1-\exp-Vx]$$

Table 7 - Comparison of Observed and Predicted TCDD-Induced Liver Tumor Rates in B6C3F1 Female Mice from the NCI Gavage Study.

Exposure ug/kg/day (X)	Number of Animals Exposed	Number (%) of Animals With Liver Tumors	
		Observed	Predicted
0	73	3 (4.1)	3.7 (5.1)
0.006	50	6 (12.0)	2.9 (5.9)
0.028	48	6 (12.5)	4.5 (9.4)
0.286	47	11 (23.4)	16.0 (34.0)
		x^2	6.46
		d.f.	3
		P value	0.09

Values of Parameters (see text)			
Symbols	Biological Interpretation	Estimates	Values drived from
G(o)	background cell growth rate	0.0533	Assumed (A priori)
$G(\infty)$	maximum cell growth rate	0.0817	Dow Study
V	incremental rate change per unit dose	13.19	NCI-Male Mice
M	background mutation rate	5.9884×10^{-7}	Estimated From Data

$$P(x) = 1-\exp-M \left\{ [\exp G(x)t-1-G(x)t]/G^2(x) \right\}$$

$$G(x) = G(o) + [G(\infty)-G(o)][1-\exp-Vx]$$

Table 8 - Comparison of Risks Between Bioassays as a Fraction of Background Rates

Sex	Species	Strain	Animal Risk at $x=1\times10^{-4}$	Background Rate	Background Rate Parameter M	Risk ÷ Background Rate	Risk ÷ M
Female	Rat	Sprague-Dawley	3.6×10^{-3}	0.1882353	2.3798×10^{-6}	1.91%	1.51×10^{3}
Female	Rat	Osborne-Mendel	7.8×10^{-4}	0.06666667	4.3036×10^{-7}	1.17%	1.81×10^{3}
Male	Rat	Osborne-Mendel	8.3×10^{-5}	0.0	4.4201×10^{-8}	---	1.98×10^{3}
Female	Mice	B6C3F1	1.3×10^{-4}	0.0410959	5.9884×10^{-7}	.32%	$.22\times10^{3}$
Male	Mice	B6C3F1	4.7×10^{-4}	0.2054795	2.6248×10^{-6}	.23%	$.18\times10^{3}$

TABLE 9

Estimation of Background Human Lifetime Liver Cancer Rate (M) and Background Human Liver Cell Proliferation Rates G(o) by Fitting $\ln[I(t)]=A+Bt=\ln(M/G(o))+G(o)t$

Age	(Mid-point)	1980 U.S. Liver Cancer Deaths ICD (155)[a]	1980 U.S. Population (thousands)	Rates/ 100,000
25-29	27.5	36	19,518	0.1844
30-34	32.5	55	17,558	0.3132
35-39	37.5	53	13,963	0.3726
40-44	42.5	95	11,668	0.8142
45-49	47.5	173	11,088	1.5602
50-54	52.5	289	11,709	2.4682
55-59	57.5	522	11,614	4.4946
60-64	62.5	699	10,086	6.9304
65-74	70	1795	15,578	11.5227
75+	80	1805	9,973	18.0970

a All races, both sexes
$\ln I(t)=-15.687+0.098381t$

Table 10 - Parameters in Human Dose Response Model

Parameters			
Symbols	Estimates		Values Derived From
	1st order	Log-Logistics	
G(o)	0.0938		Human Mortality Data
G(∞)	0.1438		Proportional to DOW Female Rats
M	1.44×10^{-8}		Human Mortality Data
V	589.16		Surface Area x DOW Female
I		19.55	DOW Female + 3 x Log Surface Area
S		3.00	Assumed

Table 11 - Estimates of Low-Dose Incremental Risk To Humans Exposed to 2,3,7,8-TCDD [a,b,c,d].

MODEL

Promotion[d]			Linearized Multistage (EPA)
Dose ng/kg-day	Negative[d] Exponential	Log-Logistic	(Upper Confidence Limit)
10^{-5}	1.7×10^{-8}	--	1.6×10^{-6}
10^{-4}	1.7×10^{-7}	--	1.6×10^{-5}
10^{-3}	1.7×10^{-6}	$<10^{-13}$	1.6×10^{-4}
10^{-2}	1.7×10^{-5}	8.8×10^{-10}	1.6×10^{-3}
10^{-1}	1.8×10^{-4}	8.8×10^{-7}	1.6×10^{-2}
1	2.4×10^{-3}	9.3×10^{-4}	1.6×10^{-1}

a $(P(x)-P(o))/(1-P(o))$

b Based on female Sprague-Dawley rats using the Squire pathology analysis and adjusting for early mortality

$$P(x) = 1-\exp-1.44\times10^{-8}\left[\frac{\exp((70)G(x)) - 1 - (70)G(x)}{G^2(x)}\right]$$

$t = 70$ years

where $G(x) = .0938+[.1438-.0938]R(x)$; $G(\infty) = (.0817/.0533)(.0938) = .1438$; and $G(o) = .0938$

$R(x) = 1-\exp(-109.5(5.38)X)$ for negative exponential

$R(x) = [1+\exp-(15.40+3 \ln 5.38x)]^{-1}$ for log-logistic

c The two forms of the two-stage model developed using the assumption that TCDD is a promoter is compared with the linearized multistage model extrapolation performed by EPA.

d With animal-to-human dose equivalence correction of $(70/0.450)^{1/3}=5.38$ based on dose/surface area.

$$P(o)=1-\exp-1.44\times10^{-8}\left[\frac{\exp(70(.0938)-1-70(.0938)]}{(.0938)^2}\right] = 1.1498\times10^{-3}$$

FIGURE 1 — GOODNESS OF FIT OF PROMOTER MODEL TO TCDD INDUCED TUMORS IN RAT LIVER

PROBABILITY OF RAT LIVER TUMOR P(x)

$$P(x) = 1 - \exp - \{ M [\exp G(x)t - 1 - G(x)t] / G^2(x) \}$$

where $t = 104$ weeks

$$G(x) = .0533 + .0284 (1 - \exp -109.5x)$$

$$M = \begin{cases} 2.38 \times 10^{-6} & \text{DOW } ♀ \\ 4.30 \times 10^{-7} & \text{NTP } ♀ \\ 4.42 \times 10^{-8} & \text{NTP } ♂ \end{cases}$$

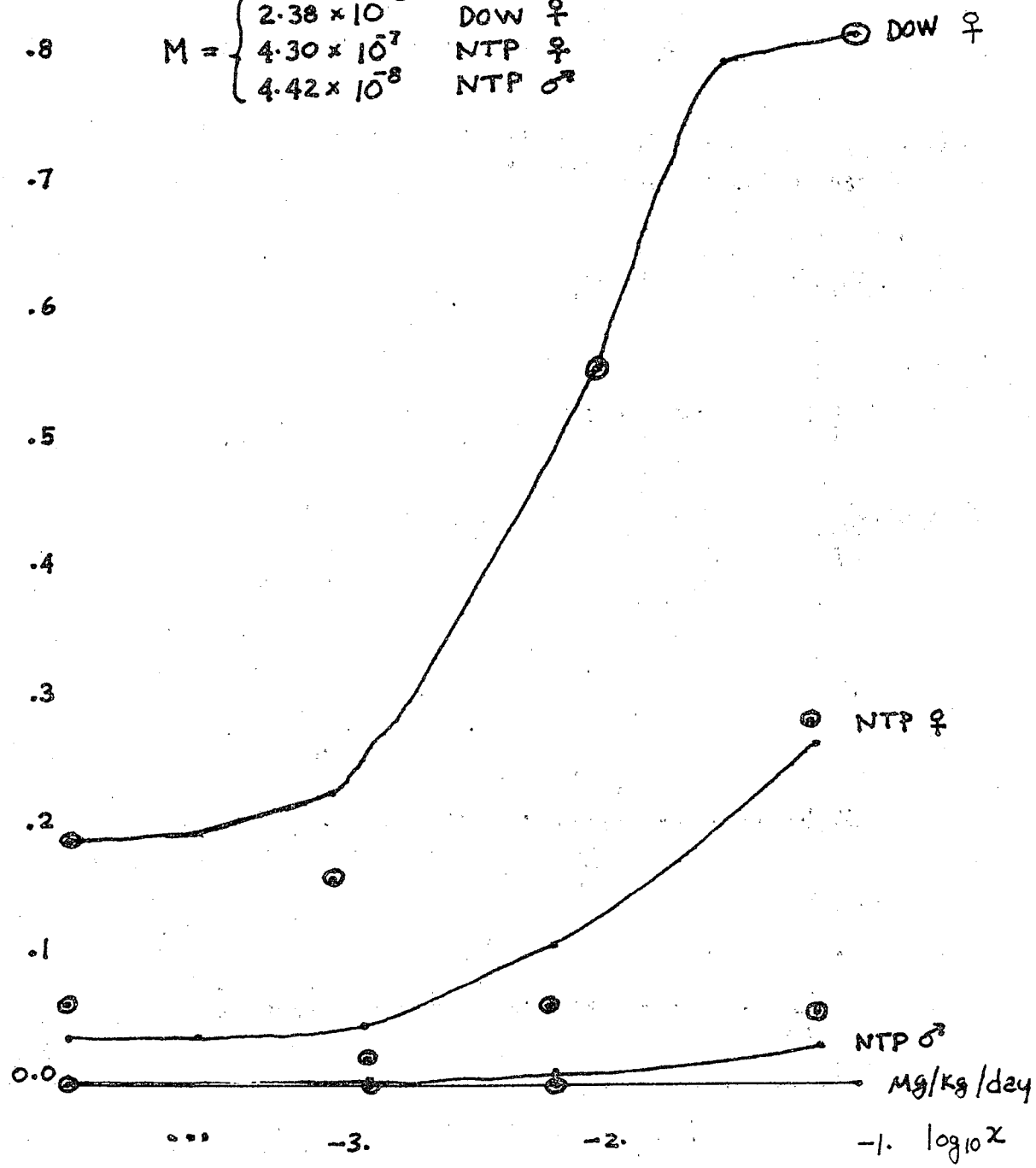


FIGURE 2 – GOODNESS OF FIT OF PROMOTER MODEL TO TCDD INDUCED TUMORS IN MICE LIVER

$$P(x) = 1 - \exp - \left\{ M \left[\exp G(x)t - 1 - G(x)t \right] / G^2(x) \right\}$$

where $t = 104$ weeks

$$G(x) = .0533 + .0284 \left(1 - \exp - 13.19x \right)$$

$$M = \begin{cases} 2.62 \times 10^{-6} & \text{NTP } \sigma^{\prime} \\ 5.99 \times 10^{-7} & \text{NTP } \varphi \end{cases}$$

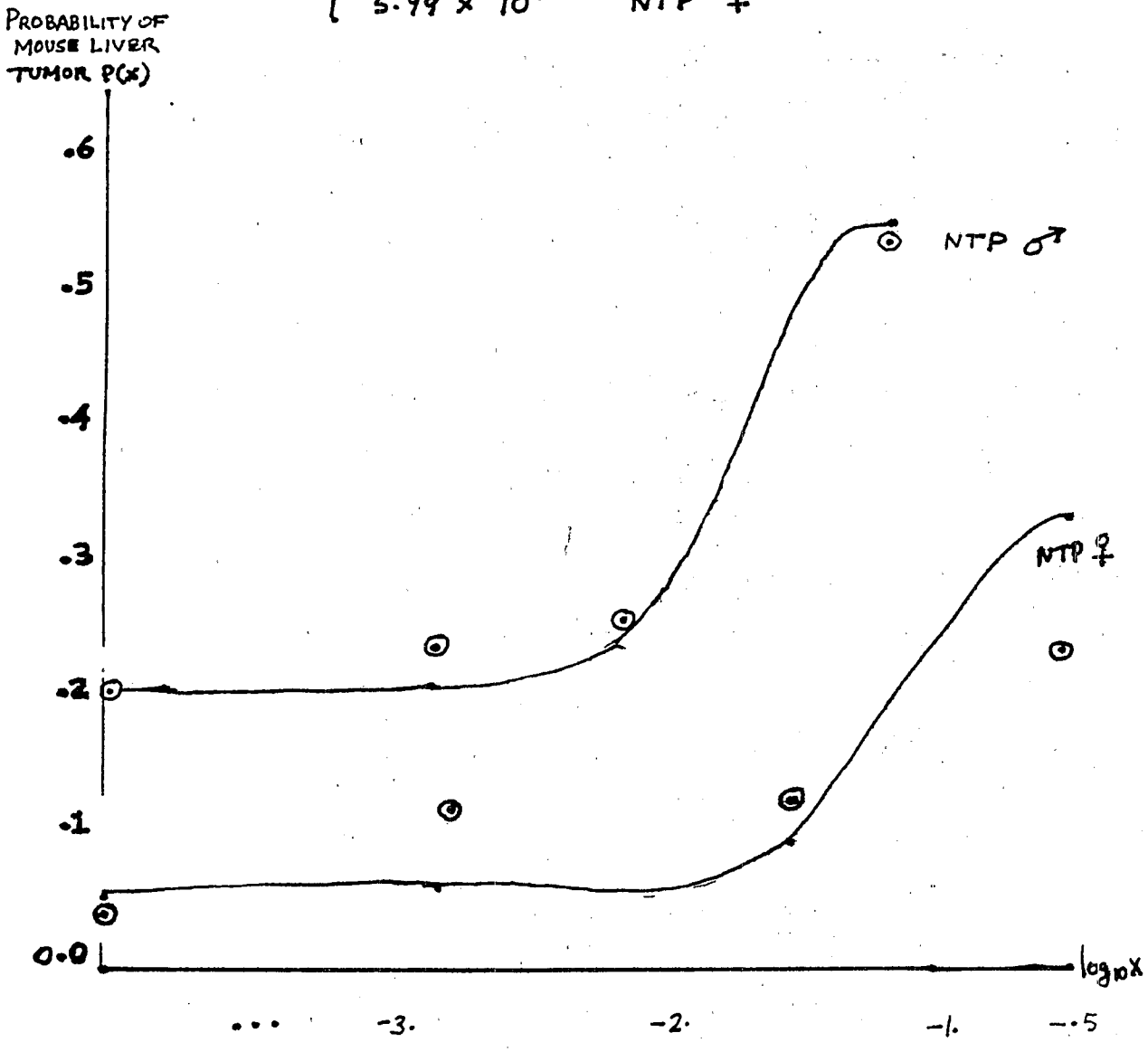
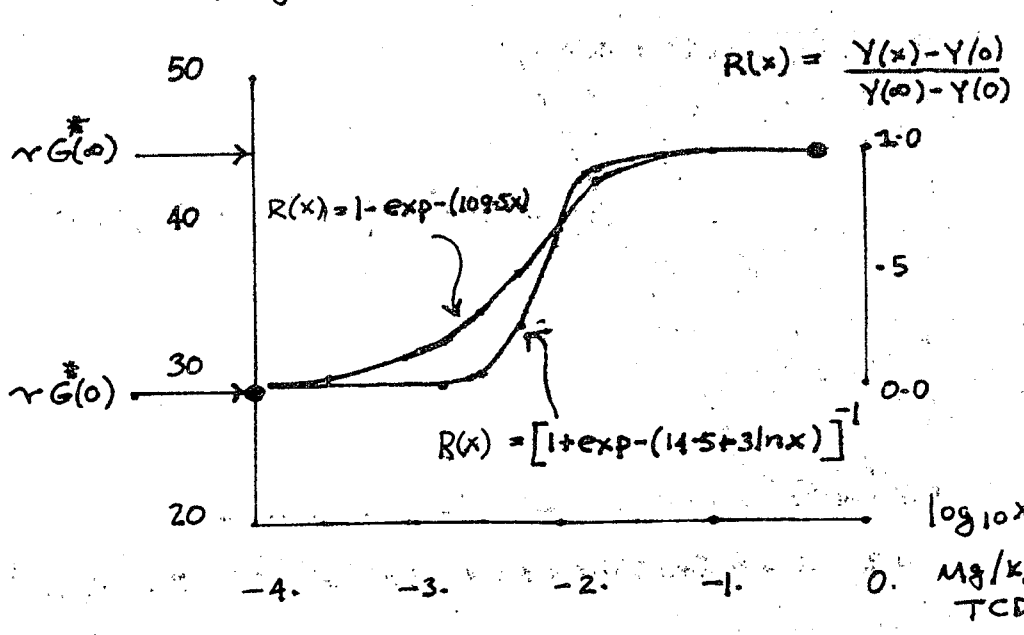


FIGURE 3- Observed and Postulated Relationships Between H^3 Thymidine Counts in Rat Liver and Exposure to TCDD

(1) H^3 count is surrogate for transient growth rate of stem cells; $G^*(\cdot)$

(2) Transient fractional change in growth rate of stem cells is same as fractional steady state change in growth rate of preneoplastic cells per unit of exposure $Y(x)$



derived from liver tumor data

$$H_0: \frac{r G^*(\infty)}{r G^*(0)} = \frac{45}{29} = 1.552 \simeq \frac{G(\infty)}{G(0)} = \frac{.0533 + .0284}{.0533} = 1.533$$

From H^3 Thymidine counts in nuclei rat liver

standard human equivalent

Figure 4 - Postulated Relationships Between H^3 Thymdine Counts in Rat Liver and Exposure to TCDD

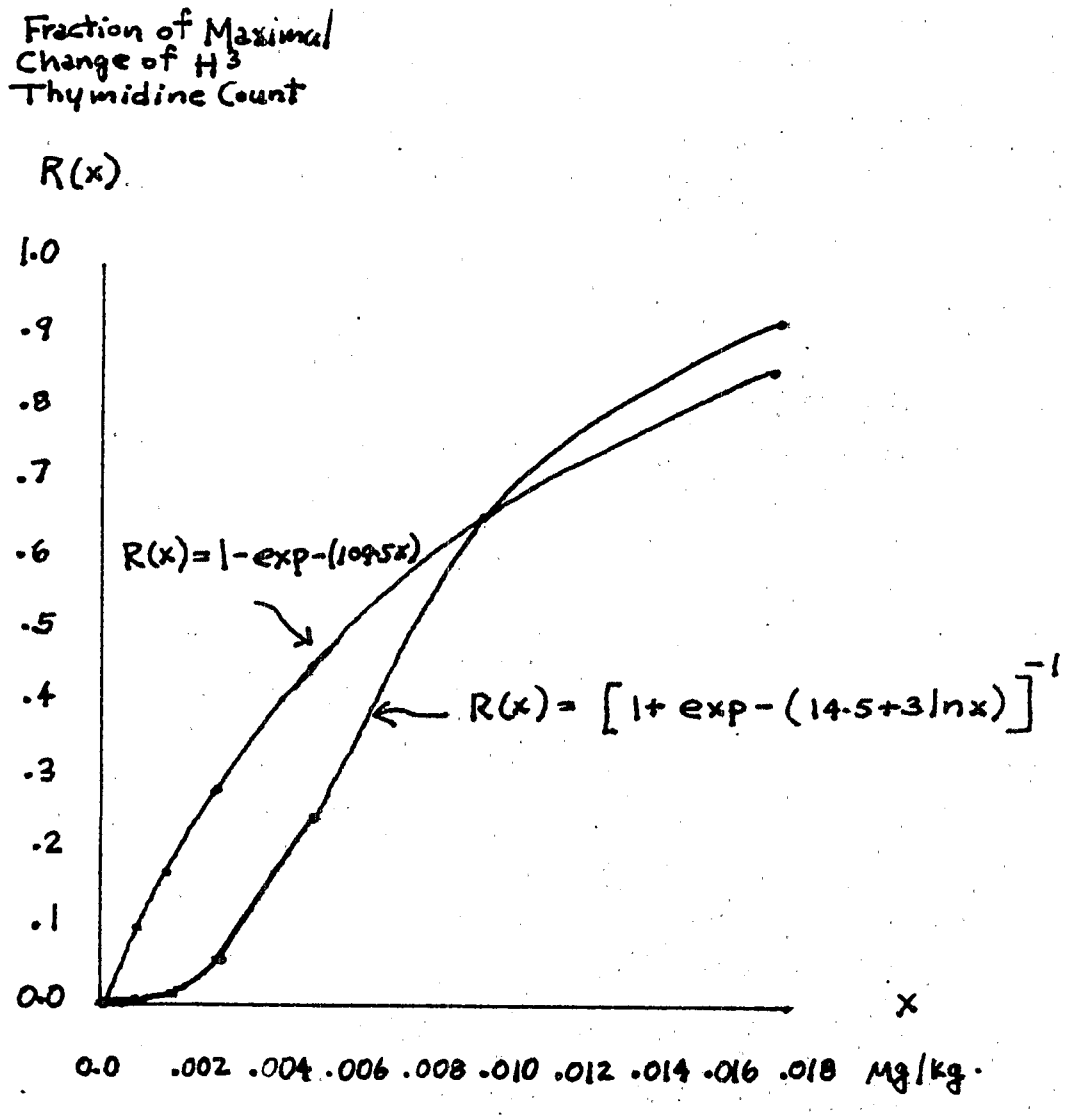
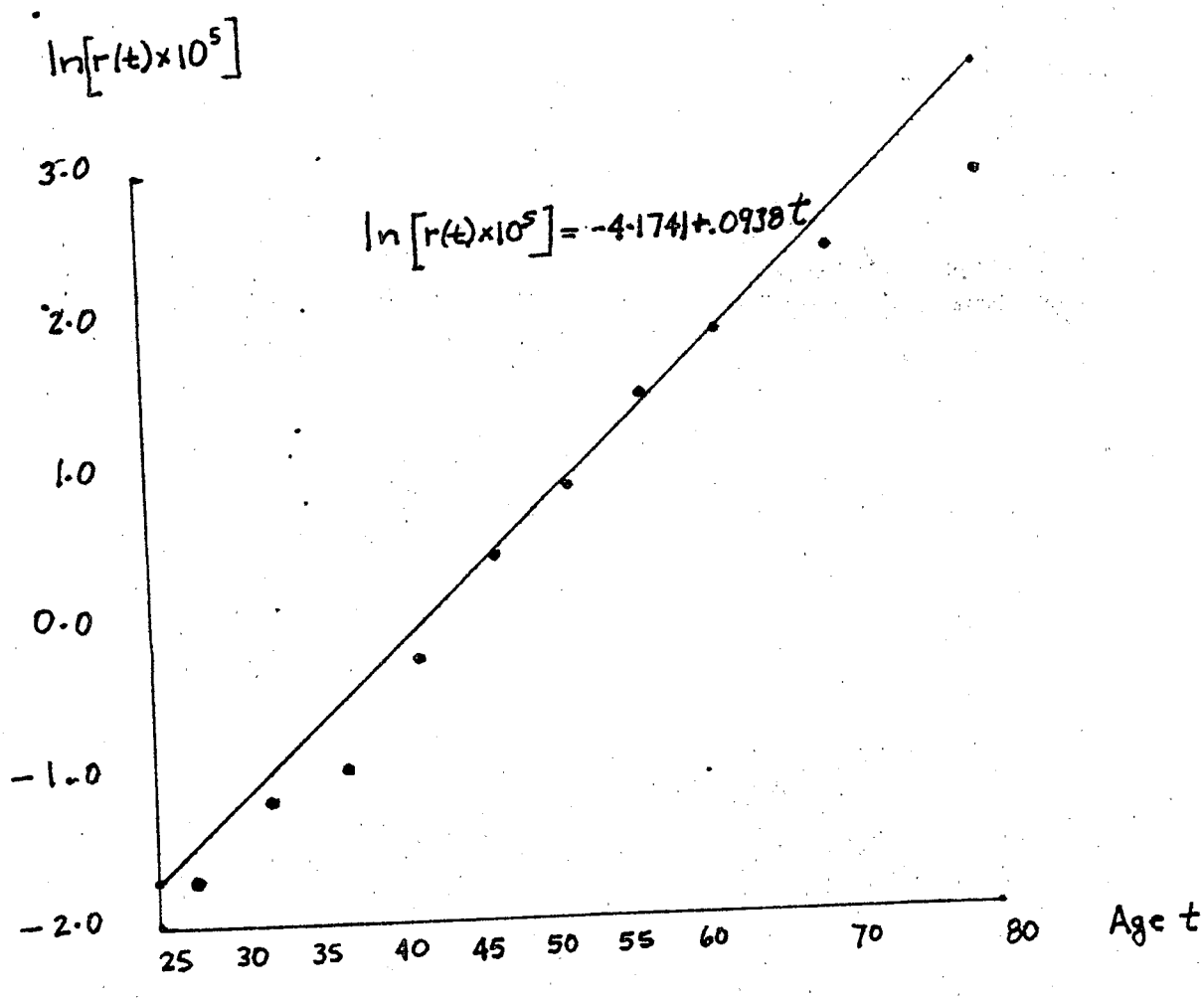


Figure 5

Relationship Between Natural Log Age Specific 1980 Total U.S. Liver Cancer Death Rates (ICD 155) and Age



$$G_H(0) \times t_h = G_A(0) \times t_a$$

70yrs human $\Longleftarrow$ 104 weeks rat

$$G_A(0) = G_H(0)\left(\frac{t_h}{t_a}\right) = .0938 \times \left(\frac{70}{104}\right) = .0631$$

References

Becker, F.F. The Direct and Indirect Effects of Promoters May Depend upon the Nature of the Initiated Cell. In: Fujiki et al. eds. Cellular Interactions by Environmental Tumor Promoters. Jap Sci. Soc. Press. Tokyo. pp. 349-349. 1984.

Christian, B.J., and Peterson, R.E. 1983. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on [^{3}H]thymidine incorporation into rat liver deoxyribonucleic acid. Toxicol. 8:133-146.

Cook, P., Doll, R., and Fellingham, S.A. (1969). A mathematical model for the age distribution of cancer in man. Int. J. Cancer, 4, 93-112.

Dickens, M., Seefeld, M.D., and Peterson, R.E. 1981. Enhanced liver DNA Synthesis in partially hepatectomized rats pre-treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 58:389-398.

Gallagher, R.E. Biochemistry of Neoplasia. In Comprehensive Textbook of Oncology, A.R. Roosa, M.C. Robson, and S.C. Schimpff, editors. Williams and Wilkins, Baltimore. Chapter 3. 1986.

Israel, D.I., and J.P. Whitlock. Regulation of Cytochrome P-450 Gene Transcription by 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Wild Type and Variant Mouse Hepatoma Cells. J. Biol. Chem. 259:5400-5402. 1984.

Jones, B.L., D.R. Galeazzi, J.M. Fisher, and J.P. Whitlock. Control of Cytochrome P-450 Gene Expression by Dioxin. Science 227:1499-1502. 1981.

Jones, P.A. DNA Methlation and Cancer. Cancer Res. 46, 461-466, February, 1986.

Kociba, R.J., D.G. Keyes, J.E. Beyer, et al. 1978. Results of a two-year chronic toxicity and oncogenicity study of 2.3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46(2): 279-303.

Moolgavkar S.H. and Venzon, D.J., "Two-Event Models for Carcinogenesis:Incidence Curves for Childhood and Adult Tumors," Math. Biosci. 47, 55-77 (1979).

Moolgavkar S.H. and Knudson, Jr., A.G., "Mutation and Cancer: A Model for Human Carcinogenesis," JNCI 66, 1037-1052 (1981).

Pitot, H.C., T. Goldsworthy and H. Poland. 1980. Promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res. 40: 3616-3620.

Poland, A. and Glover, E. An Estimate of the Maximum in vivo Covalent Bonding of 2,3,7,8-Tetrachlorodibenzo-p-dioxin to Rat Liver Protein, ribosomal RNA and DNA. Cancer Res. 39, 3341-3344. 1979.

Poland, A., and Kuntson, J.C. (1982). 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanisms of toxicity. Ann. Rev. Pharmacol. Toxicol. 22, 527-554.

Portier, C.J., Hoel, D.G., van Ryzin, J. 1983. Statistical analysis of the carcinogenesis bioassay data relating to the risks from exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin. From Public Health Risk of the Dioxins, proceedings of a symposium held October 19-20, 1983 at the Rockefeller University, New York City.

Rogers, A.M., M.E. Anderson, and K.C. Back. Mutagenicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Perfluoro-n-decanoic acid in L5178Y Mouse Lymphoma Cells. Mutat. Res. 105:445-449. 1982.

Rose, J.Q., Ramsey, J.C., Wentzler, T.H., Hummel, R.A., and Gering, P.J., 1976. The fate of 2,3,7,8-Tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36: 209-226.

Shank, R.C. Toxicity-induced Aberrant Methylation of DNA and Its Repair. Pharmacological Reviews 36(2): 195-245. 1984.

Sielken, R.L. 1986. Quantitative cancer risk assessments for TCDD. Fd. Chem. Toxicol. (in press).

Thorslund, T.W., Brown, C.C., and Charnley, G., 1987. Biologically Motivated Cancer Risk Models. Risk Analysis 7:109-119.

Upton, A.C., D.B. Clayson, J.D. Jansen, H.S. Rosenkranz, G.M. Williams. Task Group 5 Final Report: Report of the ICPEMC Task Group on the Differentiation Between Genotoxic and Non-genotoxic Carcinogens. International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) Biol. Zbl. 104 417-453. 1985.

U.S. Environmental Protection Agency (USEPA). 1985. Health Assessment Document for Polychlorinated Dibenzo-p-Dioxins. EPA/600/8-84/014F.

Weinstein, I.B. Current Concepts and Controversies in Chemical Carcinogenesis. Journal of Supramolecular Structure and Cellular Biochemistry. 17:99-120. 1981.

Weinstein, I.B. The Relevance of Tumor Production and Multistage Carcinogenesis to Risk Assessment. In D.G., Hoel, R.A. Merril, and R.F. Perera, eds. Risk Quantitation and Regulatory Policy. Cold Spring Harbor, New York. 1984.

Weisburger, J.H., and G.M. Williams. The Distinct Health Risk Analyses Required for Genotoxic Carcinogens and Promoting Agents. Environ. Health Perspect. 50:233-245. 1983.

Whitlock, J.P., D.I. Israel, D.R. Galeazzi, and A.G. Miller. 2,3,7,8-Tetrachlorodibenzo-p-dioxin Regulates Cytochrome P_1-450, Gene Expression. In Banbury Report 18. Biological Mechanisms of Dioxin Action. A. Poland, and R.D. Kimbrough, editors. Cold Spring Harbour, N.Y. 1984.

Yamasaki, J., and I.B. Weinstein. Cellular and Molecular Mechanisms of Tumor Promotion and Their Implications for Risk Assessment. In Methods for Estimating Risk of Chemical Injury: Human and Non-Human Biota and Ecosystems, V.B. Vouk, G.G. Butler, D.G. Hoel, and D.B. Peakall, editors. John Wiley and Sons, New York. 1985.

March 1988
Review Draft

APPENDIX B

EPIDEMIOLOGIC CANCER STUDIES ON POLYCHLORINATED DIBENZO-p-DIOXINS, PARTICULARLY 2,3,7,8-TCDD

David L. Bayliss
Carcinogen Assessment Group

Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

INTRODUCTION

Since the publication of the Health Assessment Document (HAD) for Polychlorinated Dibenzo-p-Dioxins (U.S. EPA, 1985), many new epidemiologic studies have been reported in the literature dealing with the risk of soft tissue sarcoma (STS) and/or non-Hodgkin's lymphoma in users and producers of phenoxyacetic acid herbicides and chlorophenols. Furthermore, the major positive studies have come under intense criticism. The earlier HAD document concluded that there was limited epidemiologic data regarding the carcinogenicity of polychlorinated dibenzo-p-dioxin-contaminated phenoxyacetic acid herbicides and/or chlorophenols based mainly on the Swedish case control studies, but the evidence regarding 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) was judged inadequate due to the inability of any of the data up to that time to demonstrate that the risk was due to 2,3,7,8-TCDD alone and not to one or more of the other 74 isomers of polychlorinated dibenzo-p-dioxins found in the phenoxyacetic acid herbicides and/or chlorophenols or the phenoxyacetic acid herbicides or chlorophenols free of 2,3,7,8-TCDD.

This report is a review and brief analysis of the epidemiologic evidence to date.

SOFT TISSUE SARCOMA

The main studies supporting the finding of an excess risk of STS were the two independent Swedish case control studies of Hardell and Sandstrom (1979) and Eriksson et al. (1979, 1981). These investigators reported statisticially significant (five- to sevenfold) elevated risks of STS from occupational exposure to phenoxyacetic acid herbicides and/or chlorophenols either alone or separately,

some of which are known to contain 2,3,7,8-TCDD as an impurity. Eriksson et al. further subdivided their cases and controls into two categories based on expected presence or absence of 2,3,7,8-TCDD among the phenoxyacetic acids after removing from the group those persons that had been exposed to chlorophenol and then recalculating the risk of STS. The relative risks were 17 in the group exposed to 2,3,7,8-TCDD-contaminated phenoxyacetic acids [2,4,5-trichlorophenoxyacetic acid (2,4,5-T)] versus 4.2 in the group exposed to other phenoxyacetic acid herbicides [(2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methyl-phenoxyacetic acid (MCPA)] not believed to contain 2,3,7,8-TCDD. This seemed to imply that either 2,4,5-T appears to be associated with a high risk of STS or the polychlorinated dibenzo-p-dioxin contaminants (such as 2,3,7,8-TCDD) within are contributing to the high risk of STS.

The Swedish studies have been severely criticized in the scientific literature because of known or alleged methodology flaws and biases. These criticisms, which are outlined in a report provided by the Australian Royal Commission (1985) on the Use and Effects of Chemical Agents on Australian Personnel in Vietnam include the following: recall bias, unreliability of the exposure data, information bias, observation bias, absence of a significant risk at any single specific cancer site, significant confounding factors, and lack of support from other studies. (Although not specifically mentioned in the report, the Eriksson et al. case-control study is included in this critique by inference.)

Some of the observations of the Australian Royal Commission have validity. Recall bias certainly may have been present. Persons who have been diagnosed with a health problem hypothesized to be associated with exposure to a given agent such as a phenoxyacetic acid herbicide are more likely to be reminded of a possible link with that herbicide than is someone diagnosed with a different health problem not hypothesized to be connected with that agent. Hence, they

are more likely to "recall" that exposure when questioned about it. However, given the rather exceptionally high risk ratios found by both investigators in their separate studies, it does not seem likely that recall bias is the explanation. At the risk ratios calculated the power exceeds 99% in both the Hardell study as well as the Eriksson study. Even if the positive responses given by the authors to the possibility of exposure were incorrect on 33% and were discarded, these studies would still retain 80% power i.e., confidence that the recalculated significant Odds Ratio (OR) measures a true association. Recall bias was discussed somewhat in the HAD for polychlorinated dibenzo-p-dioxins (U.S. EPA, 1985).

Also, there is a lack of substantiation of quantity of exposure to the phenoxyacetic acid herbicides as well as to the chlorophenols. Hardell described, in his doctoral dissertation dealing with the same cases as in his case-control study, which herbicides and chlorophenols his cases and controls were exposed to, for how long, and when they were exposed; actual quantities were not provided although latency was determined. Unfortunately, neither investigator saw a need to provide a differential analysis by latency or by quantity or length of exposure to the herbicides and/or chlorophenols. But, of course, at the time, the authors probably did not think such an analysis would be important. They did not have the vision of hindsight. Hardell and Sandstrom (1979) and Eriksson et al. (1979, 1981) contacted employers to verify exposures reported in responses to questionnaires sent to study members and survivors. The response from employers regarding use of phenoxyacetic acid herbicides was "uncertain and difficult to evaluate" due to poor record keeping by the employer. But for chlorophenol there was "good agreement" with the statements given by examined persons.

With respect to the possibility of information bias being present, this could certainly lead to an enhancement of the risk ratios as persons with STS's

(115)

would more likely assume their disease was connected with exposure to herbicides because of intense media publicity. However, in a rebuttal to the conclusions of the Australian Royal Commission, Hardell reminded the Commission that debate about phenoxyacetic acid herbicides had abated with the banning of 2,4,5-T in Sweden in 1977, thus reducing media coverage (Axelson, 1986). Hardell pointed out that the studies were undertaken during the period from 1978 to 1981.

For the purpose of assessing the possible presence of observation bias in his study, in 1981 Hardell repeated his analysis using colon cancer cases but with the same controls as in the first study. Hardell found no association between exposure to phenoxyacetic acid herbicides and/or chlorophenols and the risk of colon cancer. He pointed out that this finding is inconsistent with the occurrence of observational bias in the assessment of exposure (Hardell, 1981).

The issue of lack of site specificity with respect to the occurrence of STS is clearly not an unexpected phenomenon. Carcinogens are not always site-specific. There are many examples of multiple site carcinogens (1,3-butadiene, vinyl chloride, and arsenic to name a few). Furthermore, the human body consists of two main types of tissue, i.e., epithelial and connective. Epithelial tissue constitutes all the duct glands, skin, the GI tract, neural tube, the respiratory tract, etc. Malignant tumors originating in these tissues are called carcinomas. Connective tissue, on the other hand, consists of bone, cartilage, fat, muscle, and subcutaneous tissue. Malignant tumors originating in connective tissues are called sarcomas. With respect to appearance, a carcinoma is a hard, adhesive mass with a well-defined, advancing front. Microscopically, a carcinoma of one organ is distinguishable from that of another. In contrast, sarcomas are soft, jellylike, and fall apart easily. Microscopically, they are not clearly distinguishable by site. Corresponding to the diverse nature of

connective tissue throughout the body, sarcomas could be found anywhere in the body. They have poorly demarcated anatomic boundaries in contrast to carcinomas, and they contain large quantities of intercellular materials. As such, they are subject to the same kind of insult as is the intercellular material of epithelial tissue.

With respect to other significant confounding factors in these studies, there appears to be little substantiation of that criticism. Hardell certainly controlled for age, sex, place of birth, and place of death. He further analyzed smoking habits and found them not to be different between the cases and controls. Hardell does state, however, that other pesticides might constitute confounding factors. The Commission's statement regarding the presence of "other significant confounding factors" is a quantum leap from Hardell's commentary about his own data. This appears to consist mostly of inuendo rather than substantive criticism.

The term STS is a convenient rubric under which all sarcomas arising out of connective tissue have been classified. Individual tumor types, i.e., rhabdomyosarcoma, fibrosarcoma, liposarcoma, etc., are subtypes that identify a specialized connective tissue tumor. In the Ninth Revision of the International Classification of Diseases and Causes of Death, STS's are coded mainly to category 171x; however, under certain circumstances, they are coded to several other diffuse categories as well. If the STS is not classified to a specific site, it is given an ICD code of 171.9. If the STS is coded to a site of the body other than certain organ sites, it is still coded to the 171x category. However certain STS's of specific organ sites are coded to cancer of that site. This could serve to reduce observed STS deaths that may be due to exposure to polychlorinated dibenzo-p-dioxin and spread them out over several site-specific categories. However, since expected deaths based on population death rates in

category 171x would be subject to the same degree of difference as observed deaths, one would not expect that the calculations of relative risks would be affected although the ability to detect such elevated risks as significant would be reduced. Clearly, electron microscopy or use of immunohistological techniques are needed to distinguish a sarcoma at one site from a sarcoma at another site, but these techniques are rarely used. This difficulty in diagnosis is compounded by the fact that even collectively STS's are an exceedingly rare form of cancer in the population. Age-adjusted (1970) standard incidence rates based on category 171x during the period from 1973 to 1977 were estimated by the Surveillance, Epidemiology and End Results (SEER) group of the National Cancer Institute to be 1.9 per 100,000 in the United States (Young et al., 1982). The corresponding age-adjusted mortality rate for ICD category 171x was 0.9 per 100,000 (Table 1). STS rates generally follow the pattern of other site-specific cancer rates by age, starting off low in younger age groups (less than 2 per 100,000 in persons under 50) and increasing with age to a maximum of 12.2 per 100,000 in persons aged 85 and older. Mortality rates remain under 1 per 100,000 up to age 50 but increase gradually to a high of 7.7 in persons age 85 and older.

Additionally, the literature is replete with studies of carcinogens that cause rare cancers. These did not require confirmation before the scientific community was convinced of their authenticity (e.g., DES and vaginal clear-cell adenocarcinoma; vinyl chloride and angiosarcoma of the liver and glioblastoma multiforme of the brain).

Furthermore, although the Ninth Revision of the International Classification of Diseases and Causes of Death has assigned a specific category (171x) to this cause, it is not always certain that the correct diagnosis will be made. This was ably pointed out by Fingerhut et al. (1984) in a discussion of seven case

TABLE 1. AVERAGE ANNUAL CRUDE, AGE-SPECIFIC, AND CUMULATIVE (age 0-74) INCIDENCE AND MORTALITY RATES FOR MALIGNANT CANCER INCLUDING *in situ* CASES BY PRIMARY SITE, ALL RACES, BOTH SEXES, ALL AREAS (EXCLUDING PUERTO RICO), 1973-1977

Age group	Soft tissues (including heart)[a] Incidence	Mortality	Non-Hodgkin's lymphoma[a] Incidence	Mortality
Crude	1.9	1.0	8.9	4.9
Adjusted	1.9	0.9	9.0	4.8
Cum (0-74)	0.2	0.1	0.8	0.4
<5	1.2	1.1	0.6	0.2
5-9	0.4	0.5	0.9	0.4
10-14	0.5	0.2	1.0	0.4
15-19	0.8	0.3	1.0	0.6
20-24	0.9	0.3	1.6	0.5
25-29	1.1	0.3	2.3	0.7
30-34	1.3	0.4	2.7	1.0
35-39	1.5	0.6	3.6	1.5
40-44	1.5	0.7	6.1	2.2
45-49	1.9	0.7	8.9	4.2
50-54	2.7	1.4	13.6	6.4
55-59	3.5	1.8	18.9	9.9
60-64	4.2	2.0	27.3	13.6
65-69	4.3	2.9	34.8	19.0
70-74	7.0	3.5	45.8	27.3
75-79	8.0	4.1	51.3	34.0
80-84	9.3	4.7	53.2	39.5
85+	12.2	7.7	51.7	40.8

[a]per 100,000 population

SOURCE: Young et al., 1982.

reports of STS. Two of the seven were shown to be carcinomas. This difficulty and the exceptionally rare nature of this tumor creates methodological problems in the assessment of risk. As most trained epidemiologists are aware, the use of the cohort technique to estimate risk of rare cancers is not recommended. Even the detection of not-so-rare cancer risks requires the generation of thousands of person-years following the completion of a suitable latent period. Hence, cohort analyses of rare cancers are inappropriate vehicles for the assessment of risk. They lack power and are insensitive.

The issue of latency is of importance here. Several studies have been published that purport to show no association between exposure to 2,3,7,8-TCDD and the risk of STS as well as to most site-specific cancers. Part of the explanation for this may be the short period of time that has elapsed between onset of exposure and clinical manifestation of disease, in this case, cancer. In most nonpositive epidemiologic studies, this period has been under 10 years. This short period of time is probably inadequate to assess the risk of most site-specific cancers including STS. Few cancers have been shown to have a latent period under 10 years. Hueper and Conway (1964) suggested a latent period for the development of sarcomas of between 15 and 30 years. In the positive epidemiologic studies of Hardell and Sandstrom and Eriksson et al., the latent period has been found to range from 9 to 27 years after initial exposure to the phenoxyacetic acid herbicides and/or chlorophenols with a median time lapse of 20 years. In the Lynge (1985) study, the lapse of time from start of employment (exposure) to diagnosis ranged from 14 to 26 years if one excludes the one STS in that study with a lapsed period of time from initial employment to diagnosis of only 5 years. That is not likely to be due to exposure to 2,3,7,8-TCDD-contaminated chemicals. If one assumes that the latent period based on these positive studies is between 14 and 27 years, then

it is not likely that an excess risk of STS will appear until at least the 15th year after initial exposure. This risk should increase until around the 20th year. Probably 20 years should be considered the latent period for the development of STS from exposure to 2,3,7,8-TCDD-contaminated chemicals if one accepts the hypothesis of a causal relationship which by no means is a certainty.

It is thus remarkable that several cohort studies of individuals exposed to 2,3,7,8-TCDD-contaminated herbicides and chlorophenols have produced any STS's. And, of course, the expectation of their appearance is so small that significance tests cannot be done. The method of choice then is the case-control technique. However, the case-control technique is also frought with pitfalls, many of which are apparent in the evaluation of the epidemiology data on 2,3,7,8-TCDD. With this technique, controls are matched with actual cases usually on the basis of known confounders (i.e., age, race, sex) or suspected confounders (i.e., time of death, length of employment, and so on). Uncontrolled confounding, known or unknown, can play a significant role in the determination of a risk, as well as other factors such as latency or selection of an appropriate index of exposure if exposure cannot be directly measured. The net result can lead the researcher to attribute a significant risk, if found, to the exposure being measured instead of to a confounder which may be the real culprit. Second, if an index of exposure is used that does not truly measure the actual exposure, misclassification will result. This will force the risk estimate toward the null. Hence, if a true risk is present, it will not be found. Persons with no actual exposure could be classified as "exposed" while persons with actual exposure could be classified as "unexposed." Hence, it is always better to choose a surrogate that is as close as possible to the target organ dose. For example, human tissue levels of 2,3,7,8-TCDD would be a far better surrogate of exposure than would information that a person was located in or

near a spot where 2,3,7,8-TCDD was present.

Biases can also increase or reduce the risk calculation depending on their nature. There is really no way to control completely for some biases, i.e., recall bias and, to some extent, even information bias when dealing with questionnaire data no matter how hard the researcher might try. And these will always loom as potential problems in any case-control study, no matter how well conducted and designed it is. On the other hand, some biases could be eliminated by a suitable selection of controls. For example, if cancer controls are used, the researcher is obligated to eliminate those cancer controls where it is suspected that the cancer might be associated with the exposure under investigation. This is definitely within the power of the investigator. Based on the previous arguments there is no real basis upon which to conclude that the Hardell and Sandstrom (1979) and Eriksson et al. (1979, 1981) studies are not good scientific studies of the risk of STS.

Several other studies provided some support to the finding of an excess risk of STS among individuals exposed to 2,4,6-trichlorophenol (TCP) and/or 2,4,5-T. Zack and Suskind (1980), in a small cohort study, noted that one worker among 121 workers accidentially exposed to TCP (contaminated with 2,3,7,8-TCDD) in 1949 developed STS's. All 121 were chosen for this study because they developed chloracne which indicates substantial exposure to 2,3,7,8-TCDD. No STS's would have been expected.

Lynge (1985), in an incidence study of 3,390 males employed in two factories manufacturing phenoxyacetic acid herbicides, chiefly 2,4-D and MCPA, found a nonsignificant excess risk of STS in male employees. The author stated that these results supported the Swedish observation of an increased risk of STS following exposure to phenoxyacetic acid herbicides "unlikely to be contaminated with 2,3,7,8-TCDD." However, after a 10-year latency, the excess of STS was

significant (4 observed vs. 1.00 expected; $p < 0.05$) in male employees of the single factory where 2,4,5-T had been produced and used and where all five STS's arose. However, the author cautions that because of the limited amount of 2,4,5-T processed at that factory, exposure is unlikely although not impossible.

In a study of 2,189 manufacturing employees of a chemical plant that produced TCP, 2,4,5-T, and other chlorinated phenols contaminated with hexachlorinated to octachlorinated dioxins, Cook et al. (1986) reported one STS which was previously found by Fingerhut et al. (1984) in a case review, through pathology evaluation, not to be an STS. However, in a 1985 letter to the Carcinogen Assessment Group of the U.S. Environmental Protection Agency, Cook reported that another member of the cohort was found to be suffering from a confirmed STS. This case was not reported as an STS because the person died after the end of the follow-up period. Cook et al. did not estimate how many deaths should be expected. Latency was not considered although it appears that a sufficient follow-up had been achieved in which latency could have been evaluated for the risk of cancer at other sites. The authors believe that their data provide little evidence of a cancer risk from exposure to 2,3,7,8-TCDD especially STS.

In a later update of this same cohort by Ott et al. (1987), the authors employed a mathematical analytical technique known as a "serially additive expected dose model" designed by Smith et al. (1980). This statistical device has several limitations just as does the commonly accepted method of simply analyzing for latent effects by calculating observed and expected mortality after a lapse of a sufficient number of years from initial exposure. Despite the subjective designation of jobs according to an "intensity of exposure" scale from 0 to 4, which is not based on actual exposure and is subject to misclassification bias, it still takes 15 to 20 years for the development and

detection of a malignancy! If few persons in the cohort have achieved that latent period, no excess cancer risk will be detected no matter what the "intensity" of exposure. This method also assumes that one year of exposure at a high intensity score is equivalent to 10 years of exposure at the next lower score in its ability to produce cancer. This is not a valid assumption because such an equivalency fails to consider that a brief excursion at a very intense exposure could affect the metabolism differently from that of a long continuous (or discontinuous exposure). This device, although limited in some respects, allows for the assessment of latent effects for certain sites. Only total cancer, stomach cancer and lymphomas were analyzed for latent effects in this manner due to the "paucity of deaths through 1987." Except for total malignant neoplasms, only six stomach cancer deaths and six lymphomas were separately analyzed. The authors saw no trend of increasing risk of cancer at the few sites examined. However, it must be kept in mind that with a long latent period required for the manifestation of cancer, the power to detect a significant risk of cancer at these two sites must of necessity be small, even for this technique, although the authors did not estimate it. Of course, no other cancer sites were analyzed for latent effects by this method. Of interest is the persistently high significant risk of "other and unspecified" cancer (12 observed vs. 4.6 expected, C.I. 135-456) for which the authors could offer no explanation. This cohort or some portion of it wil be included in the National Institute for Occupational Safety and Health (NIOSH) registry of exposed workers that will be analyzed by NIOSH in the near future. One question remains, however, and that is, if 2,3,7,8-TCDD tissue levels in a substantial portion of these workers are similar to 2,3,7,8-TCDD tissue levels in the background referent population and always have been (thus indicating little evidence of differential exposure), an elevated risk of cancer would not be expected to occur. It is possible that due to the

7-year half-life of 2,3,7,8-TCDD (Pirkle et al., 1987), tissue levels that were elevated in the past from early exposure to 2,3,7,8-TCDD have returned to background levels, but of course, there is no evidence that tissue levels of 2,3,7,8-TCDD in the past were ever elevated (not always the same as they are today), and that possibly no elevation ever occurred even during times when potential exposure was hypothesized.

Hence, because of this possibility, and the knowledge that the presence of chloracne at some time in the past more than likely is proof that a substantial exposure to 2,3,7,8-TCDD occurred, it seems likely that a cohort consisting entirely of chloracne persons having known contact with 2,3,7,8-TCDD in the past would provide the most ideal cohort for assessing the long-term health effects of exposure to TCDD! This is not the same as saying that the presence of chloracne is necessary in order to have exposure. The major problem with current efforts to determine tissue levels of 2,3,7,8-TCDD is that they don't reveal what the tissue levels were in the past.

Smith et al. (1984) conducted a case-control study of STS in New Zealand and found a significantly high risk of STS among railroad workers. Specific exposure to phenoxyacetic acid could not be identified. Tannery and meat workers were also found to be at a significantly high risk of STS. In those jobs among meat workers where pelts are treated with chemicals such as TCP (2,4,6-trichlorophenol) that contain 2,3,7,8-TCDD, the risk ratio increases but is marginally significant because of small numbers. Although Smith et al. found excess nonsignificant risks in applicators and users who were exposed to only phenoxyacetic acid herbicides and/or chlorophenols, their personal exposure information is not substantiated, similar to the Swedish studies. In a later update of this study (Smith and Pearce, 1986), which included more cases and controls that had been diagnosed, the excess was reduced to the null. The

authors have been criticized by Axelson (1986) for failing to exclude from the referent group cancers that may be related to the exposure in question (i.e., lymphomas), thus potentially underestimating the true risk. Smith et al. concluded that at the levels of exposure experienced by individuals in contact with ground spraying, it is unlikely that 2,3,7,8-TCDD could cause STS.

Other studies that are consistent or are not inconsistent with the finding of an elevated risk of STS among persons potentially exposed to polychlorinated dibenzo-p-dioxin-contaminated herbicides and/or chlorophenols are Balarajan and Acheson (1984), Cantor (1982), Milham (1982), Kogan and Clapp (1985), Michigan Department of Public Health (1983a, b), Fett et al. (1984), and most recently, Puntoni et al. (1986), Merlo and Putoni (1986), Woods et al. (1987), and Kang et al. (1987). However, in many but not all of these studies, definite evidence of exposure to 2,3,7,8-TCDD is not established. Furthermore, the risk estimates are based upon generally small numbers as one might expect.

In a case-control study based on data provided by the National Cancer Register of England and Wales, Balarajan and Acheson (1984) found a significant risk of STS among farmers, farm managers, and market gardeners, but not among agricultural workers, gardeners, and groundsmen. The authors hypothesize exposure to 2,3,7,8-TCDD-containing herbicides in high risk occupational groups as a possible cause, although actual evidence of exposure is not provided.

Cantor (1982), in a case-control study, noted a significant risk of reticulum cell sarcoma among farmers under age 65 in Wisconsin in counties that were high in summary measures of General Agricultural Activity based on county-wide measures of farm-related exposures. However, occupations had been classified based on what was recorded on the death certificates. Often, the information is absent, however, or if an occupation is mentioned, it is frequently the last job held or the job of longest duration or simply the word "retired"

will appear. Such information must be considered unreliable at best and could lead to misclassification. Furthermore, the use of 2,3,7,8-TCDD-containing chemicals is only suggested by the authors.

Milham (1982), in a proportionate mortality rate (PMR) study, noted a significantly high risk of STS among farmers in Washington State. The author states that exposure would most probably have been to phenoxyacetic acid herbicides (chiefly 2,4-D) and chlorophenols. There are two major problems with this study: (1) no actual evidence of exposure is provided, and (2) the source of death information is not given. Additionally, PMR studies are inherently inferior to cohort and case-control designs.

Kogan and Clapp (1985), in a second PMR study, found a significantly high rate of connective tissue cancer (STS) among Vietnam veterans in Massachusetts as opposed to non-Vietnam veterans in Massachusetts. Again, service in Vietnam appears to be the surrogate of exposure and as such may bear no relationship to actual exposure to 2,3,7,8-TCDD-containing chemicals. It is entirely possible that many Vietnam veterans in Massachusetts were never exposed to large quantities of 2,3,7,8-TCDD-containing chemicals, while there is no reason to think that some non-Vietnam Massachusetts veterans may have been exposed to 2,3,7,8-TCDD-containing chemicals in other settings such as occupational.

The Michigan Department of Public Health (1983a, b) found significantly high death rates among females of Midland County, Michigan, compared to the national average for the period 1960 through 1978. In contrast, STS death rates for males of Midland County were not elevated during the same period. The Dow Chemical Company produced phenoxyacetic acid herbicides and/or chlorophenols in this county. Since this is an ecological study, it will of necessity include in both the numerator and denominator individuals who may or may not have received exposure to 2,3,7,8-TCDD or polychlorinated dibenzo-p-dioxin-

contaminated chemicals. Cook and Cartmill (1984), in a discussion of the soft tissue sarcoma issue, maintained that the Michigan Department of Public Health found no commonalities among the women that would suggest any single agent including exposure to 2,3,7,8-TCDD-contaminated chemicals as the cause except that several of the women moved to Midland County with pre-existing diseases. Whether the diseases they brought with them had anything to do with STS was not established. The authors also suggested that several women may have been incorrectly classified to ICD category 171x since the site of the cancer was not specifically mentioned. However, there is no reason to suspect that the Michigan Department of Public Health coded their death certificates any differently than did the National Center for Health Statistics, the U.S. agency responsible for generating U.S. and state vital statistics. These death rates provide the basis for calculating the expected deaths in that study.

A study of Vietnam veterans in Australia (Fett et al., 1984) essentially reported nonpositive findings, but the veterans also exhibited an excess of STS, albeit small (i.e., 2 observed vs. 0.64 expected). In the comparison group of non-Vietnam veterans no STS's were observed versus 0.84 expected. This study again used "service in Vietnam" as a surrogate for exposure to Agent Orange. No actual proof of exposure is provided, and one would expect many if not most of the Vietnam veterans to have had little exposure to Agent Orange.

A recent study (Puntoni et al., 1986; Merlo and Puntoni, 1986) reports a high incidence of STS in residents of the region around Seveso, Italy, where an industrial explosion at a factory that produced 2,3,7,8-TCDD-contaminated 2,4,5-T occurred on July 10, 1976. Few specifics are available regarding this study, but it is apparent that the rate of STS's was higher in residents of the Seveso region as contrasted with those in the Varese region (2 per 100,000) and tended to increase with time (i.e., 4.35 in 1981) when so-called "polluted"

areas are lumped with "non-polluted" areas. The authors suggested that these two areas be combined since chloracne rates calculated for each area separately were inconsistent with the defined soil levels of 2,3,7,8-TCDD in each area, the surrogate measure used for defining a "polluted" area as contrasted with a "non-polluted" area. Misclassification of exposure was suggested by the authors as the explanation for the lack of a positive correlation with the incidence of STS in so-called "polluted" areas. Hardell and Eriksson (1986) suggested that exposure to the chemicals produced by this factory prior to the accident accounted for the high rates before, during, and after the accident in the Seveso region since latent factors argue against the accident being the cause of the excess.

The remaining nonpositive studies are even less remarkable than those already mentioned. Thiess et al. (1982) examined a cohort of 74 employees of the Badische Anilin und Soda Fabrik (BASF) who were accidentally exposed to trichlorophenol in 1953, 66 of whom suffered chloracne; after a lengthy follow-up, no STS's appeared. However, the study has little power to detect a significant risk of cancer.

Axelson et al. (1980) studied 348 railroad workers who sprayed 2,4-D and 2,4,5-T herbicides from 1957 to 1972 and found no STS. Again, this study has limited power to detect a risk of STS as well as little evidence to substantiate exposure.

Riihimaki et al. (1982) studied 1,926 Finnish applicators of 2,4-D and 2,4,5-T from 1955 to 1971 and found no STS's (0 observed vs. 0.1 expected). However, the authors suggested major difficulties with their paper as follows ". . . an observation period under 10 years, the presence of selection bias, and low or little exposure." They conclude that it "cannot be regarded as a negative study." This study also suffers from survivorship bias.

Wiklund and Holm (1986) recently studied a massive cohort of 354,620 Swedish men who were recorded as having an agriculture or forestry job according to the census of 1960 versus 1,725,845 Swedish men in all other industries. The primary exposure in those jobs was postulated to be MCPA; 2,4-D and 2,4,5-T were also used to a lesser extent. The authors found that the relative risk of STS was only 0.9. This study has several problems: (1) a lack of individualized exposure data; (2) only 15% of Swedish agricultural and forestry workers were estimated to be exposed to phenoxyacetic acids and 2% to chlorophenols; (3) Swedish agricultural workers are known to have a decreased cancer risk and use health services less frequently; (4) classifying workers according to a 1-year employment status presents the possibility of misclassification; and (5) the crude rate of STS in agricultural and forestry workers based on data in the study is 5.45 per 100,000 person-years, and in the remaining workers it is 5.00 per 100,000 person-years. Both rates are high compared to rates from other nations (1 to 3 per 100,000 person-years).

The authors of the previous study, Wiklund et al. (1987), recently evaluated the relative risks of Hodgkin's disease and non-Hodgkin's lymphoma in a cohort of 20,245 Swedish pesticide applicators who were licensed anytime between 1965 and 1976. The authors estimate that 72% had had an opportunity for contact with phenoxyacetic acid herbicides (chiefly MCPA, mecoprop, and dichloprop, and to a lesser extent, 2,4,5-T and 2,4-D). This estimate was based on responses to a questionnaire that had been mailed to a random sample of 273 persons in the cohort. No actual measurements of exposure were taken. Overall, based on a rather short follow-up averaging 12.2 years, no significant excess risk of either disease was found. However, the authors noted a trend of increasing risks for both diseases with lapsed time since licensing. Further follow-up of this cohort is needed since presumably the latent period for lymphoma is in

excess of the observation period of this study.

Another nonpositive study (Lathrop et al., 1984a, b; Wolfe et al., 1984, 1985) of a group of young Air Force officers and skilled and unskilled enlisted men (called Ranch Handers) who presumably were involved with the aerial spraying of herbicides containing 2,3,7,8-TCDD revealed no excessive cancer risk. However, only 6 of 1,256 Ranch Handers actually developed cancer in the short period of time they were followed. Although this study offers little evidence to substantiate exposure to Agent Orange by itself, recently Pirkle et al. (1987) reported that some 75 Ranch Handers (6% of the total) had reported a range of exposure to 2,3,7,8-TCDD (from 16 to 423 ppt) based on adipose tissue samples collected by the Centers for Disease Control (CDC) and the Air Force. Furthermore, the study suffers from a lack of power and short latency.

Another nonpositive study by Greenwald et al. (1984) of 281 STS cases from the New York State Cancer Registry matched with 281 referents taken from New York driver's license files and 130 from New York death certificate files exhibited only a nonsignificant excess cancer risk for workers in chemical manufacturing and highway construction. Based on questionnaire responses, the risk from exposure to 2,3,7,8-TCDD-contaminated Agent Orange was only 0.7 while that of herbicide and/or pesticide use was 1.0. The risk for farming was 0.79. Two major problems are evident in this study. First, "service in Vietnam" was used as a surrogate for exposure to Agent Orange. This potentially can lead to a problem similar to that of other studies where exposure to Agent Orange could not be substantiated, and secondly, those who did not serve in Vietnam could have received exposure to 2,3,7,8-TCDD through other means. Furthermore, the average time lapse from Vietnam service to a diagnosis of STS in this study was only 11 years, a short latent period. Many additional years of observation must be added before the latency question can be put to rest.

Kang et al. (1986) group-matched 234 Vietnam-era veterans who were STS patients and had served in the U.S. military between 1964 and 1975 with 13,496 patients sytematically sampled from the same Vietnam-era patient population without a diagnosis of STS. Service in Vietnam (a surrogate for exposure) did not appear to be associated with STS. However, the authors reported that more than likely most patients (and controls) had not achieved a follow-up time sufficient for latent effects to become manifest. Furthermore, actual exposure as evidenced by in vivo measurements of 2,3,7,8-TCDD were not available or, as the authors pointed out, Vietnam veterans may have been exposed to such small amounts that standard epidemiologic designs could not detect an excess risk.

In a second case-control study, Kang et al. (1987) selected 217 STS patients from the Armed Forces Institute of Pathology and matched them to 599 matched controls for service in Vietnam, exposure to chemicals, medical history, and life style. Military service was verified by the patients' military personnel records. Vietnam veterans, in general, did not exhibit increased risks of STS. However, the authors found that the risk of STS increased in veterans with combat experience versus those without (OR = 2.57, nonsignificant) and increased even more for those same combat veterans who were assigned to the region where most spraying of Agent Orange took place (OR = 8.64, nonsignificant). The authors noted that their study had "very low power" to detect enhanced risks in subgroups of veterans who had greater opportunities for exposure to Agent Orange. The authors concluded that a possiblity exists that certain subgroups of Vietnam veterans may be subject to "modestly increased risks of STS" from exposure to Agent Orange, but it can neither be confirmed nor ruled out.

Karon et al. (1987, unpublished), at a recent meeting on dioxin, reported only one army veteran with a current serum 2,3,7,8-TCDD level in excess of

20 ppt out of some 775 veterans who participated in the study. About 675 of these served as ground combat troops in Vietnam and included some of the troops who presumably had had a high likelihood of exposure to Agent Orange. These levels today are not different from those found in background populations of industrialized nations. At first glance this seems to argue that veterans in Vietnam may not have been heavily exposed to Agent Orange, and hence findings based on the surrogate experience in Vietnam may not be relevant. Such may not be the case since actual time served in Vietnam occurred a considerable number of years ago. Elevated serum 2,3,7,8-TCDD levels brought on by massive exposure to Agent Orange at that time may have dissipated and returned to background levels during the period following removal of troops from Vietnam.

The Hoar et al. (1986) study is not contradictory to the hypothesis that 2,3,7,8-TCDD is the contaminant responsible for the development of STS. The authors found that 2,4-D, which does not contain 2,3,7,8-TCDD, was identified by most members of the cohort as being the herbicide to which they were exposed. Again, the results of this study are based on questionnaire data and may suffer from recall bias. This study supports only the hypothesis that the herbicide 2,4-D appears not to be a credible candidate for the carcinogenic agent responsible for STS.

NON-HODGKIN'S LYMPHOMA

Hardell et al. (1981), in a case-control study of lymphomas in Swedish workers, found a statistically significant risk of lymphoma in agricultural, forestry, and woodworking employees from exposure to phenoxyacetic acid herbicides and/or chlorophenols. Risk ratios ranged from 4.3 to 6.0 for both classes of compounds together as well as separately. However, exposure to organic

solvents such as benzene, trichloroethylene, and styrene was also found to be a risk factor. Unfortunately, the data were based on answers derived from administered questionnaires, and the exact extent of exposure to 2,3,7,8-TCDD is difficult to determine. As such, this study is subject to some recall bias and confounding, but the risk ratios are sufficiently high that problems with the study probably could not account for them entirely. This is another one of the Swedish studies that had been severely criticized for alleged biases, confounding, and distortions. The discussion following the Hardell and Sandstrom (1979) and Eriksson et al. (1979, 1981) studies regarding those criticisms pertains to this study as well.

Hoar et al. (1986), in a similar study, found significantly high rates of non-Hodgkin's lymphoma (NHL) in farmers in Kansas who use herbicides, particularly 2,4-D and triazines. Few respondents could remember exposure to 2,4,5-T which contains 2,3,7,8-TCDD. 2,4-D is not believed to contain 2,3,7,8-TCDD but does contain other polychlorinated dibenzo-p-dioxin impurities. The risk was found to increase with increasing frequency and duration of herbicide usage. Although "herbicide usage" could mean any of the herbicides identified by Hoar, she wrote that this is "essentially synonymous" with use of 2,4-D. The next most used herbicides (i.e., triazines and uracils) are nonsignificant when exposures to phenoxyacetic acids are controlled for. However, this study has problems similar to the Hardell et al. (1981) study in that there is a lack of substantiation of exposure, and the information is based on questionnaire responses that are subject to some recall bias. Moreover, there is a statistically significant risk associated with the use of other herbicides as well, i.e., triazines, amides, and trifluralin.

A population-based case-control study of the relationship of occupational exposure to the risk of STS and NHL was recently completed by Woods et al.

(1987). A total of 128 STS cases and 576 NHL cases diagnosed between 1981 and 1984 were matched with 694 randomly selected controls without cancer. The authors reported no overall increased risk of either disease from past occupational exposure to phenoxy herbicides or chlorophenols based on personal interviews. However, significant elevated risks of NHL were observed in certain subgroups thought to have somewhat heavier exposure to herbicides, i.e., farmers (OR = 1.33, CI 1.03-1.7), forestry herbicide applicators (OR = 4.8, CI 1.2-19.4), and persons potentially exposed to phenoxy herbicides for 15 or more years during the period 15 years prior to cancer diagnosis (OR = 1.71, CI 1.06-1.7). Of interest in this study is the finding that presence of chloracne is associated with a high (borderline significance, $p < 0.075$) risk of STS and less so to a risk of NHL. In so far as the information on exposure, based on questionnaire data, is somewhat suspect because actual confirmation was not obtained (i.e., adipose tissue specimens with confirmed presence of 2,3,7,8-TCDD), the presence of chloracne in conjunction with known contact with chemicals containing 2,3,7,8-TCDD could have meant a massive exposure to 2,3,7,8-TCDD above and beyond background levels. The chloracne was not clinically confirmed. Because the use of questionnaires to elicit past-history of exposure to specific phenoxy herbicides and/or chlorophenols has a potential for misclassification among cases and controls (because of memory problems and lack of confirmation of exposure), this study seems to have reported paradoxically conflicting results.

Buesching and Wollstadt (1984), in a case-control study, found that white male farmers of Winnebago County, Illinois, had a statistically significant risk of 2.65 for NHL as contrasted with all white males of that county. Although the author presents no evidence of exposure to any particular herbicide or chlorophenol, he suggested that phenoxyacetic acid herbicides were probably used. Of major concern in this study is the use of occupation as coded on the

death certificates to classify the deceased by exposure. As mentioned previously, use of such information may lead to misclassification.

Cantor (1982), in a case-control study of NHL, found a significantly high risk of NHL among farmers under age 65 in counties of Wisconsin known to be high in insecticide and herbicide use, and having small grain acreage, wheat acreage, and dairy sales. The risk of reticulum cell sarcoma is higher at 3.2 among younger farmers in these same counties. For small grain acreage and acres treated with insecticides, the risk is 6.6, and for wheat acreage, it is 4.4; all are significant. Unfortunately, information on exposure was derived based on occupations listed on death certificates similar to that of Buesching and Wollstadt (1984) and as such cannot be considered reliable. The potential for misclassification of exposure among the cases and controls is great. Additionally, no particular herbicide, insecticide, or pesticide was mentioned. They were only surmised to be in use by the author.

Burmeister et al. (1983), in another case-control study of NHL in Iowa farmers, found a statistically significantly high risk of NHL associated with farming. It was found to be elevated in those born before 1901 and was associated with jobs involving egg-laying chickens, solid milk products, hog production, and herbicide use. This study, which also depended on occupation as recorded on death certificates for exposure, may be very unreliable, and it is likely that misclassification could have resulted. The authors suggested that herbicides and/or insecticides, such as those mentioned in the Hardell and Sandstrom (1979) study, may be carcinogenic.

Milham (1982), in a proportionate mortality ratio study of deceased workers in the agriculture, forestry, and wood products industries of Washington State, noted a significant excess of Hodgkin's disease and a high but nonsignificant risk of NHL in paper and pulp mill workers. Again, there is no evidence of

actual exposure to phenoxyacetic acid herbicides and/or chlorophenols cited by the authors. Furthermore, the proportionate mortality design is inherently inferior to the cohort or case-control study design because the former forces interdependence of the risk estimates.

Cook et al. (1986), in a cohort study of 2,189 manufacturing employees of a chemical plant that produced TCP, 2,4,5-T, and other chlorinated phenols found a nonsignificant increased risk of NHL (5 observed deaths versus 2.1 exposed). However, the authors reported that these results do not support a causal association between chronic disease and exposure to the chemicals in question. The problems with this study involve lack of evidence of exposure to most members of the cohort (only 15% had chloracne), and no effort was made to assess latent effects.

Ott et al. (1987) updated the Cook et al. (1986) study by using a statistical technique known as a "serially additive expected dose model" that assesses latent effects. Based on six lymphomas, no latent trend was found. The problems with this design were discussed previously.

Other studies, such as those of Lynge (1985), Zack and Suskind (1980), Thiess et al. (1982), Axelson et al. (1980), Riihimaki et al. (1982), Lathrop et al. (1984a, b), Wolfe et al. (1984), Fett et al. (1984), and Kogan and Clapp (1985), did not find an excess risk of NHL in individuals who were potentially exposed to 2,3,7,8-TCDD-contaminated chemicals. All of these studies have methodological flaws that make it unlikely that they could detect a significant risk if one were present. The Lynge (1985) study noted only a slight excess of lymphomas in males after a lapse of 10 years from initial exposure; however, sensitivity was somewhat reduced. The Zack and Suskind (1980), Thiess et al. (1982), and Axelson et al. (1980) studies were exceptionally small studies and hence lacked sensitivity because of their size despite a lengthy follow-up.

The Riihimaki et al. (1982) study, although seemingly large, had many methodological deficiencies as described in the section under soft tissue sarcomas that precluded detection of a risk of NHL. The study by Lathrop et al. (1984a, b) and Wolfe et al. (1984) of Ranch Handers also lacked sufficient statistical power to detect a risk as being significant. Furthermore, there was a lack of evidence of substantiation of differential exposure to Agent Orange in this study, a short latent period in which to expect the development of NHL, and a lack of sensitivity for the detection of NHL. The Australian Veterans Health Studies (Fett et al., 1984) are much like the Ranch Handers studies in that they lack sensitivity, provide little evidence of differential exposure, and have a short latent period. Kogan and Clapp (1985) did not evaluate NHL in their study of Massachusetts veterans. This study also had several limitations which were discussed in the section on soft tissue sarcomas.

STOMACH CANCER

Two small cohort studies by Thiess et al. (1982) and Axelson et al. (1980) reported statistically significant excesses of stomach cancer (albeit three cases in each study), while a third smaller study (121 persons) by Zack and Suskind (1980) reported no excess risk of stomach cancer. This exceptionally small cohort lacks sufficient power. Only 0.5 stomach cancer deaths would have been expected to have occurred by the end of the cut-off date in that study. Lynge (1985) reported a nonsignificant excess risk of stomach cancer in chemical workers in Denmark. Cook et al. (1986) noted a slightly elevated risk of stomach cancer in his cohort which was also nonsignificant. Cook et al., however, did not analyze their data for latent effects. The Riihimaki et al. (1983) cohort study is another nonpositive study with large deficits of mortality. This

study suffers from major problems not the least of which is a survivorship phenomenon. The members of this cohort had to live long enough to be included in the cohort. In the Wolfe et al. (1984) and Lathrop et al. (1984a, b) study of Ranch Handers, no excesses of any cancer of any kind were found. Again, this mortality study of mostly young men produced only six cancer deaths to date, and has several problems as mentioned previously. Burmeister et al. (1983), in a case-control study, noted a significant risk of stomach cancer in individuals who had jobs in "milk products, cattle production, and/or jobs involving high yields of corn per acre." The authors suggested as a cause possible exposure to herbicides and/or insecticides such as those reported in the Hardell and Sandstrom (1979) study. However, occupation as recorded on death certificates was the surrogate used by the authors for exposure, and as such is a very unreliable vehicle for the determination of exposure.

ALL OTHER CANCERS

Other cancer sites have been found to be significantly elevated among persons or groups potentially exposed to 2,3,7,8-TCDD-contaminated chemicals. Buesching and Wollstadt (1984) and Burmeister et al. (1983) reported significant elevated risks of prostate cancer in farmers in Winnebago County, Illinois, and agricultural workers in Iowa, respectively. Both used death certificate designation of occupation as their surrogate for exposure. As was pointed out earlier, occupation as recorded on death certificates is very unreliable. No substantiation of exposure to any herbicides or pesticides was done.

Milham (1982) found a significant excess of Hodgkin's disease in persons employed in the agriculture, forestry, and wood products industry of Washington State. His source of data was not revealed. However, there is again no evidence

actual exposure to 2,3,7,8-TCDD-containing phenoxyacetic acid herbicides and/or chlorophenols in this proportionate mortality study.

Kogan and Clapp (1985), in his proportionate mortality study of Vietnam veterans versus non-Vietnam veterans in Massachusetts, found a significant excess of kidney cancer. Again, no evidence of actual exposure to Agent Orange was given. This study and its methodological flaws were discussed previously. In short, service in Vietnam is the surrogate for exposure.

Finally, Cook et al. (1986) found a statistically significant excess of other and unspecified cancers in his study of employees of a chemical plant that produced TCP, 2,4,5-T, and other chlorinated phenols. Again, the flaws in this design were discussed previously.

SUMMARY AND CONCLUSIONS

The evidence that persons who are exposed to phenoxyacetic acid herbicides and/or chlorophenols are subject to an elevated risk of STS's is based primarily on two independent case-control studies by Hardell and Sandstrom (1979) and Eriksson et al. (1979, 1981). The problems with these studies, as outlined in the section on STS, are not sufficient to explain the highly significant risks of STS found in workers exposed to these chemicals. Power considerations alone indicate that as many as 1/3 of the positive responses in the cases could be reversed without a major loss of power in both studies. Additionally, Eriksson's data further suggest that the risk is greater from exposure to 2,3,7,8-TCDD-contaminated herbicides such as 2,4,5-T rather than from those herbicides free of 2,3,7,8-TCDD contamination. Several additional studies tended to support or were consistent with the Hardell and Sandstrom (1979) and Eriksson et al. (1979, 1981) findings of an excess risk of STS in certain subgroups of the study

groups thought to be more highly exposed to 2,3,7,8-TCDD-contaminated chemicals (Zack and Suskind, 1980; Lynge, 1985; Balarajan and Acheson, 1984; Cantor, 1982; Milham, 1982; Kogan and Clapp, 1983; the Michigan Department of Public Health, 1983a, b; Puntoni et al., 1986; Merlo and Puntoni, 1986; Wood et al., 1987; and Kang et al., 1987). However, except for the Zack and Suskind study, where proof of substantial exposure to 2,3,7,8-TCDD-contaminated trichlorophenol is evident by the occurrence of chloracne in all subjects, differential exposure to 2,3,7,8-TCDD, sufficient to produce a detectable change in risk estimates, can only be assumed to have occurred where proof of exposure is available. In addition, authors of several studies have maintained that their own results do not support the results of the Swedish studies. However, many of these studies exhibit small nonsignificant risks of STS when it could be measured. For example, the Cook et al. (1986) study produced one STS where none were expected, while the Smith et al. (1984) study produced a significant risk of STS in workers involved in treating pelts with chlorophenols. Even the Fett et al. (1984) study of Australian Vietnam veterans produced two STS's where only 0.67 were expected. On the other hand, in studies where no risk of STS was found, such as Thiess et al. (1982) and Axelson et al. (1980), the cohorts are exceptionally small, and one would not expect to see any of the exceedingly rare STS's even though many years have passed. Even the defective study of Riihimaki et al. (1982) anticipated only 0.1 STS. Wolfe and Lathrop's Ranch Handers exhibited little cancer mortality, and never achieved a latent period long enough to detect any STS's. Wiklund and Holm (1986), on the other hand, in their massive study of STS in Sweden, although nonpositive, provided enough data to calculate crude STS rates that appear unusually high compared to that of other countries. Woods et al. (1987) found a borderline significant risk of chloracne although clinically unconfirmed with risk of STS. Kang et al. (1987) found a high risk

of STS in Vietnam veterans most likely to have been exposed to Agent Orange, although power was very low. Greenwald et al. (1984) used "service in Vietnam" as a surrogate for exposure. Only five of his nine "exposed" Vietnam service cases actually thought they were exposed to Agent Orange, while four "non-Vietnam service" controls claimed exposure to phenoxyacetic acid herbicides in occupational settings. The Hoar et al. (1986) study seems to imply only that 2,4-D and triazines could be ruled out as a cause of STS, since 2,3,7,8-TCDD-contaminated 2,4,5-T was believed not be present. Hence, this study does not really contradict the Swedish studies because 2,4,5-T was not considered. Most of these studies suffer from one or more methodological flaws that preclude any conclusion that they are nonsupportive of a finding of a risk of STS.

The net result is that basically the Swedish studies of Hardell and Sandstrom and Eriksson et al. provide the best evidence of a causal relationship from exposure to the phenoxyacetic acid herbicides and/or chlorophenols. There is some additional support from some small cohort studies and a few poorly and not so poorly designed case-control and proportionate mortality studies. However, these two Swedish case-control studies, contrary to the criticism leveled at them, appear to have been constructed and executed along classic lines as outlined in any good epidemiology course on methodology. The authors appear to have been concerned about possible bias, confounders, and other problems in their analyses as well as their critics. Hardell and Sandstrom re-examined their data by using colon cancer cases and matching them with the same controls in order to assess the effect of certain biases with which they were concerned. Under any other circumstances these studies would be considered reasonably good studies minus certain analyses relating to dose response that probably were not envisioned at the time by the authors. Largely on the basis of these Swedish studies, the International Agency for Research on Cancer decided to reclassify

the phenoxyacetic acid herbicides and/or chlorophenols in 1986 as probable human carcinogens based on limited evidence of their carcinogenicity in humans.

Although the epidemiologic data appear to provide limited evidence that exposure to phenoxyacetic acid herbicides and/or chlorophenols is causally related to the risks of STS, none of these studies could be said to incriminate 2,3,7,8-TCDD directly as the agent solely responsible for the excess of STS's. The question remains that the risk of STS may be in part due to the presence of other polychlorinated dibenzo-p-dioxin contaminants as well or even to the phenoxyacetic acid herbicides and/or chlorophenols themselves, or perhaps some unknown confounder not heretofore discovered. Hence, based on the epidemiologic data, the evidence still must be considered inadequate but suggestive that 2,3,7,8-TCDD is carcinogenic to humans.

With respect to non-Hodgkin's lymphoma (NHL), a similar situation exists. Data from Hardell et al. (1981) and more recently Hoar et al. (1986) support the findings of an excess risk of NHL from exposure to phenoxyacetic acid herbicides and/or chlorophenols. However, this risk is confounded by the presence of organic compounds also shown to be carcinogenic on their own in the Hardell et al. study. In the Hoar et al. study, although 2,3,7,8-TCDD-contaminated 2,4,5-T was not identified as being present, other herbicides and insecticides as well as 2,4-D were present and were significantly associated with exposure. The contaminant 2,3,7,8-TCDD appears not to be present in any of these compounds in the Hoar et al. study although other isomers of polychlorinated dibenzo-p-dioxin were present. Wiklund et al. (1987) noted a trend of increasing risks for Hodgkin's disease and NHL with lapsed time since licensing of herbicide applicators although nonsignificant. Woods et al. (1987) reported significantly increased risks of NHL in certain occupational subgroups known to have a high likelihood of contact with herbicides. Buesching and Wollstadt

(1984) found a significant excess of NHL in deceased farmers in Winnebago County, Illinois, but this study used occupations as listed on the death certificates as the basis for determining exposure. No particular herbicide or other chemical was identified however. Cantor (1982) noted a similar significant excess of NHL (reticulum cell sarcoma) in young Wisconsin farmers in counties with a high summary measure of agricultural activity, such as number of acres in small grain acreage, number of acres treated with insecticides, and number of acres in wheat acreage. Again, no particular herbicide, pesticide, or chlorophenol was identified. Burmeister et al. (1983) also noted a significant excess of NHL in Iowa farmers who were involved with "egg-laying chickens, solid milk production, hog products, and herbicide use." Again, no particular herbicide was identified. Similarly, Milham (1982) noted, in a proportionate mortality study, a higher nonsignificant risk of NHL in Washington State paper and pulp mill workers. Cantor (1982) stated that 2,4-D and/or chlorophenols were chiefly used in these industries.

Cook et al. (1986), in his cohort mortality study, also reported a non-significant excess risk of NHL in chemical workers exposed to trichlorophenols, 2,4,5-T, and 2,4,5-T formulations.

Other studies that report slight excesses of NHL or none at all include: Zack and Suskind, Thiess et al., Lynge, Axelson et al., Riihimaki et al., Fett et al., Wolfe et al., and Kogan and Clapp. However, these studies cannot be said to support the argument that there is no risk because of problems connected with each of them, i.e., exceptionally small cohorts, insufficient latency, insensitivity, and, in some instances, little evidence of exposure. These problems were discussed previously.

The net overall thrust of the data supports the judgment that there is limited human epidemiologic evidence that the phenoxyacetic acid herbicides

and/or chlorophenols are causally related to NHL. However, with respect to identifying any particular isomer, such as 2,3,7,8-TCDD, it must be regarded as inadequate.

With respect to stomach cancer, Thiess et al. (1982) and Axelson et al. (1980), in small cohort studies, found significantly high stomach cancer risks based on three cases in each study. Zack and Suskind (1980), on the other hand, did not find any in another small study. Only one other study produced a significant excess of stomach cancer, and that is the case-control study by Burmeister et al. (1983) in which the surrogate for exposure was occupation as given on the death certificates. No other study reported a signficant excess of stomach cancer, although most studies have methodological flaws. At this time, the data must be considered only suggestive at best that phenoxyacetic acid herbicides and/or chlorophenols cause stomach cancer in humans based on the epidemiologic data. Again, with respect to the potential carcinogenicity of 2,3,7,8-TCDD, the data must be regarded as inadequate.

Other excess cancer risks by site occur sporatically at best in a few of these epidemiologic studies. They must be regarded as inconclusive. A summary of the preceding text will be found in tabular form in Tables 2 through 8 following the section on Future Expectations.

ONGOING RESEARCH

Several agencies of the U.S. government are conducting ongoing research that may help to resolve some of the issues raised regarding interpretation of the human health studies on 2,3,7,8-TCDD. NIOSH is conducting both a morbidity and mortality study of workers potentially exposed to TCP, 2,4,5-T, and pentachlorophenol, and therefore 2,3,7,8-TCDD. Using NIOSH-registry data, which

includes demographic and work history information on some 7,000 employees of 14 different production plants in the United States where potential exposure to 2,3,7,8-TCDD may have occurred, NIOSH is conducting a historic prospective mortality study. The results will be reported in the near future.

The second major use of the registry data will be to conduct a hands-on medical study of some 450 workers at two of the 14 plants. Some 450 referents were age, sex, race, and neighborhood-matched based on Census block information. Noninvasive medical tests (including blood specimens) will be conducted in order to evaluate presence or absence of health conditions hypothesized to be associated with exposure to TCP, 2,4,5-T, and pentachlorophenol and consequentially to 2,3,7,8-TCDD. Only the first phase of this study is complete. That phase included complete evaluations of approximately 80 cases and their referents. Two papers are in preparation at this time; they include an assessment of chloracne in pentachlorophenol workers as well as a description of medical results.

The IARC and National Institute of Environmental Health Sciences are jointly maintaining an international registry of persons occupationally exposed to phenoxy acids, chlorophenols, and contaminants during their manufacture and use. This study will pool together cohorts from over 12 countries. The same protocol will be used, and the data will be analyzed as one big study. The NIOSH registry is included as part of this study.

The CDC is further conducting pilot exposure studies on former Vietnam veterans in conjunction with the Veterans Administration (VA). Currently, blood is being drawn from some 600 Vietnam and non-Vietnam veterans and analyzed for the levels of 2,3,7,8-TCDD. This information will be used to compare and validate categories of exposure that have been defined in advance regarding the degree of exposure to 2,3,7,8-TCDD sustained by these veterans.

Additionally, CDC and the VA are corraborating on a study of about 40 "heavily" exposed Ranch Hand veterans to determine the half-life of 2,3,7,8-TCDD in blood. Apparently, new blood will be drawn and the levels of 2,3,7,8-TCDD will be determined in that new blood and contrasted with the levels of 2,3,7,8-TCDD found in old blood drawn from the same persons at an earlier time. This project was expected to be complete in September of 1987.

CDC is also planning a series of case-control studies of liver cancer, nasal cancer, STS, and lymphomas in Vietnam veterans to be completed in 1989.

The National Cancer Institute (NCI) is planning a case-control study of Nebraska farmers exposed to phenoxyacetic acid herbicides similar to the Hoar et al. (1986) study of Kansas farmers. Results will be available in 1989.

FUTURE EXPECTATIONS

The NIOSH studies should provide answers to whether U.S. workers involved in the production of herbicides and chlorophenols contaminated with 2,3,7,8-TCDD suffer from increased risks of site-specific cancer mortality as well as other long-term health effects a priori found to be associated with exposure to 2,3,7,8-TCDD. The CDC studies will help to substantiate or invalidate subjectively defined 2,3,7,8-TCDD exposure gradients in Vietnam veterans as well as provide estimates on the half-life of 2,3,7,8-TCDD in blood. This information will be useful in estimating quantitatively the probable dose received by Vietnam veterans at the time of exposure to Agent Orange. A series of case-control studies planned by CDC on Vietnam veterans of cancer sites shown previously to be associated with a higher risk of exposure to Agent Orange may provide additional evidence to either refute or substantiate a higher risk of cancer, especially if it can be determined what the likely dose was at the time

of exposure based on the half-life estimates. The NCI case-control study of Nebraska farmers should serve as a check on the results of the earlier Hoar et al. (1986) study of Kansas farmers.

TABLE 2. CASE STUDIES--2,3,7,8-TCDD

Author(s)	Exposure	Years worked	D_x	Cancer type	Chloracne	Major concerns
Cook (1981)	Trichlorophenol (TCP); operator, plant mechanic (TCP department).	1951 to 1982 (19 years)	?	Malignant fibrous histiocytoma	Yes	Died after the cut-off date of Cook's later study of 2,189 men in 1986.
Moses and Selikoff (1981)	Maintenance and service worker for 32 years in a chemical manufacturing site that produced TCP and 2,4,5-T; first exposed to 2,4,5-T in 1948 or 1949.	1943 to 1975 (32 years)	1980	Neurogenic sarcoma	No	This person would have been expected to work throughout the plant in maintenance.
Johnson et al. (1981)	2 patients: father and son--both exposed to chlorophenols.	Father: 1951 to 1980	1980	Liposarcoma	No	
		Son: 1978 to 1980 (but exposed 4 years earlier to phenols)	1980	Fibrosarcomatous mesothelioma	No	Short latency period.
Sarma and Jacobs (1981)	3 patients: First: member of helicopter rescue unit of USAF--defoliants.	First: defoliants 1966 and 1967, while on rescue missions.	1979	Fibrous histiocytoma	?	Definite exposure to 2,3,7,8-TCDD or polychlorinated dibenzo-p-dioxin not proven.
	Second: U.S. Marine, stationed in areas where defoliants were used; completely soaked with Agent Orange, twice.	Second: Vietnam in 1966 and 1967.	1979	Mediastinal fibrosarcoma	?	
	Third: U.S. Army, stationed in areas of Vietnam where defoliants were used.	Third: 1971	1981	Pleural/diaphragmatic leiomyosarcoma	?	Definite exposure to 2,3,7,8-TCDD not proven.

(continued on the following page)

TABLE 2. (continued)

Author(s)	Exposure	Years worked	D_x	Cancer type	Chloracne	Major concerns
Bishop and Jones (1981)	First: Pentachlorophenol manufacturing.	1959 to 1972	1976	Non-Hodgkin's lymphoma (NHL)	Yes	Exposed to other chemicals, including aromatic hydrocarbons such as benzene
	Second: Operator in the same plant.	1957 to 1978	1978	Non-Hodgkin's lymphoma	Yes	Exposed to other chemicals, including aromatic hydrocarbons such as benzene.
		Length of exposure	Age at death			
Olsson and Brandt (1981)	First: Lumberjack exposed to 2,4,5-T and 2,4-D.	20 years	40	Non-Hodgkin's lymphoma	Presence of cutaneous lesions	This study is really a case-control study of 123 men with a recent D_x of NHL.
	Second: Gardener and farmer, MCPA and insecticides.	18 years	45	Non-Hodgkin's lymphoma	Cutaneous lesions	The authors found a significant association of cutaneous lymphoma with exposure to phenoxy acids, as follows:
	Third: Lumberjack, 2,4,5-T and 2,4-D.	20 years	57	Non-Hodgkin's lymphoma	Cutaneous lesions	
	Fourth: Farmer, MCPA and 2,4-D.	18 to 20 years	64	Non-Hodgkin's lymphoma	Cutaneous lesions	

NHL		Occupational exposure to phenoxy acids	Absence of same
	With cutaneous lymphoma	4	1
	Without cutaneous lymphoma	7	111

$OR = 63.4$
$X^2 = 23.8$ ($p<0.001$)

(continued on the following page)

TABLE 2. (continued)

Author(s)	Exposure	Years worked	D_x	Cancer type	Chloracne	Major concerns
May (1982)	79 workers developed chloracne following exposure to tetrachlorodibenzodioxin due to an accident on April 24, 1968. Workers were re-examined 10 years later.	No deaths or cancers were reported.				Length of follow-up for determination of carcinogenic effects has not been achieved in this study.
Pazderova-Vejlupkova et al. (1981)	80 workers engaged in production of 2,4,5-T between 1965 and 1968 became ill. The majority developed chloracne, while 11 manifested porphyria cutanea tarda. Workers not lost to follow-up were re-examined for only a few years and for not more than 10 years. Length of follow-up was not mentioned.	Only 6 deaths occurred: 2 brochogenic carcinomas 1 arteriosclerosis 2 traffic accidents 1 hepatitis				Length of follow-up for determination of carcinogenic effects has not been achieved in this study.

TABLE 3. EPIDEMIOLOGIC CANCER STUDIES OF POLYCHLORINATED DIBENZO-p-DIOXINS, PARTICULARLY 2,3,7,8-TCDD: CHEMICAL WORKERS

Author(s)	Exposure	Salient points	Major findings	Major concerns
Zack and Suskind (1980)	Accidental exposure to trichlorophenol (TCP) as the result of an industrial accident in 1949. All colort members developed chloracne.	Occurrence of even one extremely rare soft tissue sarcoma (STS) (1975) in this small cohort of workers having sufficient latency and proof of exposure is important.	Total/Deaths: 121/32 Site — Obs/exp Lung — 5/2 STS — 1/-	The ability of this study to detect an effect as statistically significant, if the effect is truly present, is poor. This study lacks power.
Thiess et al. (1982)	Accidental exposure to TCP containing 2,3,7,8-TCDD at a BASF factory in Ludwigshafen, FRG, in 1953. Of 74 persons studied, 66 suffered chloracne or severe dermatitis.	After 10 years' latency, a significant excess of stomach cancer appears. This study provides good evidence of exposure and sufficient latency.	Total/Deaths: 74/31 Site — Obs/exp Stomach — 3/0.52 ($p<0.05$)	This study has limited statistical power to detect an effect if the effect is truly present, especially STS.

(continued on the following page)

TABLE 3. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Lynge (1985) (incidence)	Employees of 2 factories involved in the manufacture of phenoxy herbicides 2,4-D and 4-chloro-2-methyl-phenoxyacetic acid (MCPA) prior to 1982, unlikely to be contaminated with 2,3,7,8-TCDD. However, 2,4,5-T was produced at one of the factories.	The author maintains that the finding of a nonsignificant excess risk of STS supports the Swedish observation of an excess risk of STS following exposure to phenoxy herbicides unlikely to be contaminated with 2,3,7,8-TCDD. A significant excess risk of STS (4 obs, 1.00 exp; $p<0.05$) at the single factory where 2,4,5-T was produced or used following a latent period of 10 years.	Total/Cancer cases Males 3,390/159 Females 1,069/49 Site — Obs/exp STS 5/1.84 (males); 0/0.75 (females) Lymphomas 7/5.37 (males); 1/1.21 (females) Stomach 12/9.32 (males); 1/1.47 (females) Lung 38/31.80 (males); 6/2.71 (females) STS 4/1.00 (males) ($p<0.05$) (after 10-year latent period at one factory)	Exposure assessment was not made. Departmental categories were used to stratify cohort. Although the author maintains that the exposure encountered by the members of the cohort was only to 2,4-D and MCPA, he presents data that 2,4,5-T was also produced at the same factory where all five STS's were found. Latent period is too short in one case. No confirmation of actual exposure was made to any chemical.

(continued on the following page)

TABLE 3. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Cook et al. (1986)	Manufacturing employees of a chemical plant that produced TCP, 2,4,5-T, and other chlorinated phenols. Chloracne occurred in about 15% of the group. Although presence of chloracne implies substantial exposure to dioxin in this group, persons without frank chloracne (85%) may also have been exposed.	The authors stated that the results of their study do not support a causal association between chronic disease and exposure to chlorinated phenols, derivative products, etc. However, excesses occur across several cancer sites suggested as possible target sites for cancer, albeit nonsignificant. Since STS's can be classified to several different sites, it would appear plausible to expect increases of cancer risk at these sites, albeit nondetectable. Latency analysis might sharpen the risk estimates.	Total/Deaths 2,189/298 Site — Obs/exp Stomach 5/3.2 Lung 18/22.0 Male genitals 6/3.8 Connective 1/- Non-Hodgkin's lymphoma 5/2.1 Other and unspecified 9/3.7 ($p<0.05$)	Although a sufficiently lengthy follow-up period is evident, the authors did not stratify the data according to latency. Although the one connective tissue death was later found to be a clear cell carcinoma, one additional employee of this plant, who was reported to have frank chloracne, was diagnosed as having an STS and subsequently died of it after the cut-off date for this study. The remaining 85% without frank chloracne may have sustained exposure to 2,3,7,8-TCDD that was little different than that of the comparison population. Hence, no increased risk may be detectable.

(continued on the following page)

TABLE 3. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Ott et al. (1987)	Same cohort as Cook et al. (1986)	Authors saw no trend of increasing risk of total cancer, stomach cancer, or lymphomas. Cancer of other and unspecified remains significantly elevated.	Total/Deaths 2,192/370 Site — Obs/exp Stomach 6/3.8 Respiratory system 23/27.9 Male genitals 8/4.8 Connective and other STS 1/0.4 Non-Hodgkin's lymphoma 5/2.6 Other and unspecified 12/4.6 ($p<0.05$)	Mathematical analytical technique known as a "serially additive expected dose model" allowed the authors to check latency on only three cancer sites. None of the remaining cancer sites were so analyzed. Because of the small number of deaths for stomach cancer (6) and for lymphoma (6), the power to detect a risk must, of necessity, be small even after a long latent period. Intensity of exposure scale consists of five categories rated 0-5. Category five is highest. This is very a subjective designation.

(continued on the following page)

TABLE 3. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Puntoni et al. (1986) and Merlo and Purtoni (1986)	Industrial explosion at an Italian factory that produced 2,3,7,8-TCDD-contaminated 2,4,5-T on July 10, 1976, plus possible exposure to polychlorinated dibenzo-*p*-dioxin pollutants emmitted from this plant earlier than the time of the accident.	High incidence of STS in residents of region in Italy known as Seveso.	Age-adjusted STS incidence rates (per 100,000) Combined "polluted" and "unpolluted": Cases / Rate 1975: 7 / 2.71 1976: 7 / 2.88 1977: 7 / 2.84 1978: 10 / 3.86 1979: 6 / 2.29 1980: 11 / 4.04 1981: 11 / 4.35 1975-81: 59 / 3.34 By comparison, in the Varese region of Italy, the rate of STS is 2 per 100,000.	Few specifics available. Latent factors argue against the accident being responsible for increased incidence in Seveso region. "Polluted" areas around Seveso show flat incidence rates over the same period of time, while "non-polluted" areas show increasing incidence with time. Authors suggested that definitions of "polluted and "non-polluted" areas are incorrect and misclassification of cases may have take place. Authors suggested that prior pollution to polychlorinated dibenzo-*p* dioxin prior to the accident may be responsible for increasing the rate over the whole region.

TABLE 4. EPIDEMIOLOGIC CANCER STUDIES OF POLYCHLORINATED DIBENZO-p-DIOXINS, PARTICULARLY 2,3,7,8-TCDD: USERS AND APPLICATORS

Author(s)	Exposure	Salient points	Major findings	Major concerns
Axelson et al. (1980)	Swedish railroad workers who sprayed herbicides containing polychlorinated dibenzo-p-dioxin along tracks from 1957 to 1972. Herbicides used were amitrol, 2,4-D, 2,4,5-T, plus other organic and inorganic herbicide preparations.	After 10 years' latency, a significant excess of stomach cancer appeared.	Total/Deaths: 328/45 Site: Stomach — Obs/exp: 3/0.71 ($p<0.05$)	This study has limited statistical power as well as poor information about types of phenoxy herbicides present and concentrations to which individuals were exposed. No other confirmatory evidence of exposure, i.e., chloracne, was presented.
Riihimaki et al. (1982, 1983)	Herbicide applicators of four Finnish employers involved with brushwood controls. Briefly exposed to 2,4-D and/or 2,4,5-T from 1955 to 1971.	No conclusions regarding the presence or absence of a cancer risk can be derived.	Total/Deaths: 1,926/144 Site: Lymphomas — Obs/exp: 0/0.8 Site: STS — Obs/exp: 0/0.1	Although the cohort seems large, the observation period was only 8 years, beginning in 1972. However, only those applicators who were alive in 1972 were included in the cohort, although to qualify for inclusion they had to have worked a minimum of 2 weeks sometime during 1955 to 1971. A total of 45 individuals who died prior to 1972 were excluded. This is a survivorship cohort.

(continued on the following page)

TABLE 4. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Riihimaki et al. (1982, 1983) (continued)				The authors themselves suggested the presence of selection bias, an insufficient latent period, and low exposure (short duration). The authors concluded that it "cannot be regarded as a conclusive negative study."
Buesching and Wollstadt (1984)	Deceased farmers of Winnebago County, Illinois, exposed to fertilizers and pesticides. Standard comparison population was with white male county residents. Person-years were estimated by linear extrapolation of age-specific Census Bureau figures.	A significant elevated risk of non-Hodgkin's lymphoma (NHL). Authors suggested a common risk to farm-related exposures.	Total/Cancer deaths ?/1,733 Site — Obs/exp NHL 2.65 ($p<0.05$) Prostate 1.95 ($p<0.05$) Leukemia 2.00 STS and stomach cancers were not evaluated by the authors.	No evidence is presented identifying exposure to 2,3,7,8-TCDD, although it is known that phenoxy herbicides were probable used. Use of occupation and industr as recorded on death certificates is a very unreliable vehicle to identify exposure and will likely lead to misclassification of exposure.

(continued on the following page)

TABLE 4. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Balarajan and Acheson (1984)	Farmers, agricultural workers, and rlated occupational groups. Determined among STS cases recorded by the National Cancer Register in England and Wales. Job title is a surrogate for exposure.	Risk of 1.7 in group comprising farmers, farm managers, and market gardeners.	84 STS cases. Comparison population is population from census of England and Wales (1971). Risk = 1.7 ($p = 0.05$) in farmers, farm managers, and market gardeners. Risk = 1.0 in agricultural workers. Risk = 0.7 in gardeners and groundsmen.	No evidence of exposure to any agent in question is given. The authors hypothesize exposure to a common carcinogen within the three high risk categories. However, it is unlikely that farmers, farm managers, and market gardeners would be exposed to herbicides, insecticides, etc., and agricultural workers, gardeners, and groundsmen would not.
Fett et al. (1984) Australian Vietnam Veterans Healths Study--Mortality	The surrogate for exposure is "service in Vietnam."	Slight excess of STS in servicemen who spent time in Vietnam.	Service / Total/Deaths Vietnam 19,209/260 Non-vietnam 26,957/263 Site/Total cancer / Obs/Exp Vietnam 31/32.9 Non-Vietnam 41/43.0 STS Vietnam 2/0.64 Non-Vietnam 0/0.84 NHL Vietnam 3/2.04 Non-Vietnam 4/2.67	Australian servicemen did not participate in herbicide application, and Australian ground troops were less likely to be in areas where Agent Orange was used than were U.S. troops. They were young and relatively healthy, with low mortality. The nonpositive findings do not show a correlation between herbicide exposure and specific causes of death. Maximum latency only 16 years.

(continued on the following page)

TABLE 4. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Wiklund and Holm (1986)	Cohort consisted of 354,620 Swedish men who were agricultural or forestry workers according to the population and housing census of 1960. They were contrasted with 1,725,845 Swedish men employed in other industries. Exposure was primarily to 4-chloro-2-methyl-phenoxyacetic acid (MCPA). MCPA is not considered to be contaminated with 2,3,7,8-TCDD. 2,4-D and 2,4,5-T were used to a lesser extent.	The author concluded that despite the "greatly increased use of phenoxyacetic acid herbicides from 1947 to 1970, no time-related increase in the risk of soft tissue sarcoma was found in the total cohort ." It is interesting to note, however, that based on a rate of 2 STS per 100,000 persons per year found in the U.S., the Swedish number of STS is more than 2 times greater than that expected in the U.S. based on total person-years provided in both the cohort and the referent group.	Total/Cases of STS 354,620/331 Site / Obs / Relative Risk STS / 331 / 0.9	The major problem with this study is the lack of individualized exposure data in either the study cohort or the referent cohort. The authors estimate that Swedish agricultural workers have been shown to be a group with a decreased risk of cancer in general, and they used health services less that other occupational groups. Finally, the possibility of misclassification of the members of both cohorts based on 1 year's employment status also could reduce sensitivity, although the authors feel this is small. Latency was not looked at in this study.

(continued on the following page)

TABLE 4. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Wiklund et al. (1987)	Swedish pesticide applications licensed between 1965-1976. Only 72% had an opportunity for contact with phenoxy acid herbicides chiefly MCPA, mecoprop, dichlorprop, and, to a lesser extent, 2,4,5-T and 2,4-D based on questionnaires sent to 273 members of the cohort.	Although authors saw no significant risk of either NHL or Hodgkin's disease, they did note a trend of increasing risks for both diseases with lapsed time of licensing.	Total/Cases of STS 20,245/? Site — Obs — Relative Risk NHL — 21 — 1.01 Hodgkin's — 11 — 1.20	No measurements of exposure, potential only. Average follow-up time was only 12.2 years. Not enough time for detection of latent effects. Authors recommend continued follow-up.

(continued on the following page)

TABLE 4. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Wolfe et al. (1984)	Air Force personnel known as "Ranch Handers," who were involved in aerial spraying of herbicides in Vietnam, were characterized as having exposure to 2,3,7,8-TCDD containing herbicides.	No STS's were found. Largest cause of death was that of accidents.	<u>Total/Deaths</u> Cohort - 1,256 Ranch Handers/ 54 deaths Comparison - 6,171 cargo mission and support personnel/ 265 deaths Only 6 Ranch Hander deaths were attributable to cancer, while 43 comparison group deaths were due to cancer. None of these were STS. No excesses were noted.	This study lacks sufficient power to detect an excess risk as significant. It is basically a study of young men. Very little mortality has occurred to date, except accidents. Few biological markers (such as chloracne) were evident in this group to substantiate exposure. In fact, most of the "Ranch Handers" were officers and specialized enlisted men who might not be expected to have extensive exposure to 2,3,7,8-TCDD-contaminated herbicides. The South Vietnamese did much of the spraying around air bases. They might constitute a better study cohort. Pirkle et al. (1987) recently reported that 75 Ranch Handers (6% of the total) provide adipose tissue specimens with levels of 2,3,7,8-TCDD ranging from 16 ppt to 423 ppt. Some of these are considered high.

(continued on the following page)

TABLE 5. CASE-CONTROL STUDIES OF POLYCHLORINATED DIBENZO-p-DIOXINS, PARTICULARLY 2,3,7,8-TCDD: SOFT TISSUE SARCOMAS

Author(s)	Exposure	Salient points	Major findings	Major concerns
Hardell and Sandstrom (1979)	Applicators of phenoxyacetic acid herbicides and chlorophenols in the agricultural and forestry industries known to be contaminated with polychlorinated dibenzo-p-dioxin, mainly 2,3,7,8-TCDD. Patients were selected from patients of the Department of Oncology, University of UMEA, and administered questionnaires.	Risk of STS is exceptionally high. Power exceeds 99%.	Soft tissue sarcomas 52 STS 206 referents Risk was 5.3 - pH only ($p<0.05$) Risk was 6.6 - CL only ($p<0.05$) Risk was 5.7 - Combined ($p<0.05$)	Exposure to the agent(s) in question is not substantiated. It is based mainly on recall of patients and referents, although some effort was made by the authors to substantiate exposure through contact with employer, whenever possible. Possibly some recall bias is present.
Eriksson et al. (1981)	Applicators and users of phenoxyacetic acid herbicides and chlorophenols in the forestry and agricultural industries in southern Sweden. Cases were patients with STS diagnosed and reported to cancer registry of the National Social Welfare Board, 1974 to 1978. Information on exposure came from use of questionnaires.	Risk of STS is exceptionally high. Risk ratios considerably greater for 2,3,7,8-TCDD-contaminated phenoxyacetic acid herbicides (OR = 17.0) compared to 2,3,7,8-TCDD-free phenoxyacetic acid herbicides (OR = 4.2). Power exceeds 99%.	Soft tissue sarcomas 110 STS 219 referents Risk was 6.8 - pH only ($p<0.05$) Risk was 3.3 - CL only ($p<0.05$) Risk was 4.7 - Combined ($p<0.05$)	Exposure to the agent(s) in question is not substantiated. It is based mainly on recall of patients and referents, although some effort was made by the authors to verify reported employment through contact with employers, whenever possible. Possibly some recall bias is present.

(continued on the following page)

TABLE 5. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Smith et al. (1984)	Cases were selected from male cases reported to the National Cancer Registry of New Zealand. Referents were other cancer cases from same registry. Questionnaires were administered to identify occupations and industries involved in use of phenoxy herbicides and chlorophenols.	Risk of STS was significantly high in railway workers who sprayed chemicals (OR = 3.2). It was significantly high in workers in meat works (OR = 2.8), especially in the tannery or meat works' pelt department (OR = 7.2) from exposure to TCP.	Soft tissue sarcomas 112 STS (82 actual) 112 referents (92 actual) Risk was 1.6 - pH only (more than 5 years earlier) Risk was 1.5 - CL only (more than 5 years) Risk was 1.3 - pH (more than 10 years earlier) Risk was 1.6 - CL only (more than 10 years) Risk was 3.2 ($p<0.05$) - pH (railway workers) Risk was 2.8 ($p0<.05$) - CL (meat works) Risk was 7.2 - CL (tannery or meat works' pelt department)	Exposure to the agent(s in question is not substantiated. It is based on recall of exposure as determined from questionnaires administered to cases and referents. Authors used other cancer cases as referents without removing those cancers that may have been related to the exposure in question, thus leading to a possible dilution of risk estimates. One-to-one matching of cases with referents is no longer valid due to loss of 30 cases and 20 referents.

(continued on the following page

TABLE 5. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Greenwald et al. (1984)	Data on cases and referents were gathered from N.Y. State Cancer Registry. Live referents were chosen from N.Y. driver's license files. Dead referents were taken from N.Y. State death certificate data. Questionnaire data included questions about military service experience, work in occupations or industries with herbicide exposure (chemical manufacturing, highway maintenance, etc.) and agricultural farming.	No direct association of STS was found with military service in Vietnam, military service in general, exposure to Agent Orange, "dioxin," or 2,4,5-T, farming, herbicide or pesticide usage, etc. Only work in chemical manufacturing and highway construction (OR = 1.77 and OR = 1.50, respectively) demonstrated a nonsignificant elevated risk.	281 cases 281 live referents 130 dead referents OR = 1.77 (chemical manufacturing) OR = 1.50 (highway construction) OR = 0.70 (Agent Orange, dioxin, or 2,4,5-T) OR = 1.0 (herbicide and/or pesticide) OR = 0.79 (farming)	Information is based responses to questior naires and, as such, respondents may have been unable to recall exposure to the agent in question, when in fact exposure occurre "Service in Vietnam" was used as the surrc gate for exposure to Agent Orange. No actual evidence of exposure was given. Only 5 of 9 "exposed' cases believed they were exposed to Agent Orange, while 4 "non-exposed" controls claimed exposure to phenoxy herbicides ir occupational setting: Furthermore, a short latent period is evident in which to expect the develop-ment of STS.

(continued on the following pa

TABLE 5. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Hoar et al. (1986)	Farmers in Kansas who use herbicides, particularly 2,4-D and triazines. Few respondents remembered exposure to 2,4,5-T. Cases were identified through the University of Kansas Cancer Data Service. Referents were identified from the general population of Kansas.	No increased risk of STS was found from use of farm herbicides. 2,3,7,8-TCDD not shown to be present in 2,4-D.	200 STS cases OR = 0.9 for farm herbicide use	Same as mentioned in the Hoar et al. (1986) study under the section on non-Hodgkin's lymphoma.

(continued on the following page)

TABLE 5. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Woods et al. (1987)	Occupational exposure to phenoxy herbicides or chlorophenols based on personal interviews.	No increasing risk of STS was associated with overall duration or intensity of chemical exposure or with exposure to any specific phenoxy herbicide. However, an excess borderline significant risk of chloracne was found in those workers reporting STS.	Soft tissue sarcomas 128 STS 694 referents Risk was 0.80 phenoxy herbicides Risk was 0.99 chlorophenols Risk was 1.77 herbicide applicators Risk was 1.55 planer mill worker Risk was 4.83 log-lumber inspector Risk was 2.66 lumber graders ($p<0.05$) Risk was 3.32 presence of chloracne ($p<0.075$) Risk was 1.72 skin blisters from chemicals	No measurements of actual exposure were taken--all questionnaire responses. Possible slippage by age when cases were group matched. The controls overall were older than were the cases. The authors claim they took care of age as a confounding variable by "age-adjusting by 5- or 10-year age groups, where possible." This is rather vague. Possible misclassification of exposure because of use of questionnaire to elicit past history of exposure.

(continued on the following page)

TABLE 5. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Kang et al. (1986)	Previous military service in Vietnam and presumably exposure to Agent Orange	No significant association of STS and previous military service in Vietnam.	STS 234 STS 13,496 referents Risk was 0.83 overall	Insufficient latent period for observation of latent effects. Actual exposure information not available. Previous military service in Vietnam may bear no relationship to actual exposure.
Kang et al. (1987)	Occupational and non-occupational exposure to various chemicals, Vietnam service, medical history, and life-style.	Vietnam veterans in general did not have an increased risk of STS when compared to non-Vietnam veterans. However, subgroups of Vietnam veterans who have higher estimated opportunities for Agent Orange exposure seem to be at greater risk of STS.	STS 217 STS 599 referents Risk was 0.85 overall Risk was 2.57, nonsignificant, veterans with combat experience Risk was 8.64, nonsignificant, combat veterans located where Agent Orange was sprayed.	No measurements of actual exposure. No power to detect elevated risks in subgroups possibly exposed to Agent Orange. Possibly an insufficient latent period was used to observe latent effects.

TABLE 6. CASE-CONTROL STUDIES OF POLYCHLORINATED DIBENZO-p-DIOXINS, PARTICULARLY 2,3,7,8-TCDD: LYMPHOMAS

Author(s)	Exposure	Salient points	Major findings	Major concerns
Hardell et al. (1981)	Users of phenoxyacetic acid herbicides and chlorophenols in the agricultural, forestry, and woodworking industries known to provide an opportunity for exposure to 2,3,7,8-TCDD. Patients were selected from the Department of Oncology, University of UMEA. Patients and respondents were asked which phenoxy herbicides and chlorophenols they were exposed to.	67% of patients had non-Hodgkin's lymphoma (NHL). 33% had Hodgkin's lymphoma. Highly significant risk of NHL from exposure to phenoxyacetic acid and chlorophenols.	Lymphomas 169 lymphomas 338 referents Risk was 4.8 - pH ($p<0.05$) Risk was 4.3 - Chlorophenols ($p<0.05$) Risk was 6.0 - Combined ($p<0.05$)	Exposure to organic solvents such as benzene, trichloroethylene, and styrene also occurred and produced an increase in the risk of lymphoma. In fact, the author concluded that "exposure to organic solvents, chlorophenols, and/or phenoxyacetic acids constitute a risk factor for malignant lymphoma." Exact extent of exposure to 2,3,7,8-TCDD contaminants was not substantiated. Based on questionnaire responses, the data are subject to recall bias and observation bias.

(continued on the following page)

TABLE 6. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Cantor (1982)	Frequency of farming occupation. Exposed were defined to be those coded from death certificates, i.e., farmer (owner, tenant, or foreman), using 1960 U.S. Census definition.	Risk increases with length of follow-up. Significant elevations of odd ratios for reticulum cell sarcoma were observed among farmers under age 65 in Wisconsin counties high in insecticide use, herbicide use, acerage of small grains, wheat acreage, and dollars of dairy sales. Author maintains that risk begins to increase temporally with use of agricultural chemicals, among them chlorinated phenoxyacetic acids.	Non-Hodgkin's lymphoma 774 NHL deaths 774 referents (deaths from other causes) Farming in general: . Risk = 1.22 Farming for persons under age 65: Risk = 1.7 ($p<0.05$) Risk of reticulum cell sarcoma higher at 3.2 among younger farmers in counties with high summary measure of general agricultural activity and of small grain acreage and acres treated with insecticides = 6.6; and of wheat acreage = 4.4.	Death certificates are very unreliable for occupational determination. There are no individual personalized exposures. Not even phenoxyacetic acid or chlorophenols were identified as being used; such use is only surmised.

(continued on the following page)

TABLE 6. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Burmeister et al. (1983)	Exposed were defined to be those white males over 30 years of age for whom an agricultural job was recorded on their death certificates in Iowa between 1964 and 1978.	Non-Hodgkin's lymphoma was associated with egg-laying chickens, milk products sold, hog production, and herbicide use. Stomach cancer was associated with milk products sold, cattle production, and corn per acre. Authors suggested as one possible cause, exposure to 2,4-D or 2,4,5-T insecticides. There is a common etiological thread running through three of the cancers (multiple myeloma, non-Hodgkin's lymphoma, and leukemia), although the authors did not look at leukemia.	Cases Multiple myeloma - 550 Non-Hodgkin's lymphoma - 1,101 Prostate cancer - 4,827 Stomach cancer - 1,812 Controls: 2 per case Farming - Risks Multiple myeloma - 1.48 ($p<0.05$) Non-Hodgkin's lymphoma - 1.26 ($p<0.05$) Prostate cancer - 1.19 ($p<0.05$) Stomach cancer - 1.32 ($p<0.05$)	Death certicates are very unreliable for occupational determination. Furthermore, there is no substantiation of exposure to any of the pesticides (herbicides), as suggested by the authors.

(continued on the following page)

TABLE 6. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Hoar et al. (1986)	Farmers in Kansas who use herbicides, particularly 2,4-D and triazines. Few respondents remembered exposure to 2,4,5-T. Cases were identified through the University of Kansas Cancer Data Service. Referents were identified from the general population of Kansas.	Significant dose-response relationship of non-Hodgkin's lymphoma with frequency and duration of herbicide usage. Authors claim this is synonymous with use of 2,4-D. No association of STS or Hodgkin's lymphoma with herbicide usage. 2,4-D is not contaminated with 2,3,7,8-TCDD.	Cases Hodgkin's lymphoma - 173 Non-Hodgkin's lymphoma - 200 Referents: 1,005 (3 per case) Risks Hodgkin's lymphoma - 0.8 Non-Hodgkin's lymphoma - 1.4 Non-Hodgkin's lymphoma - 1.6 (herbicide use) Non-Hodgkin's lymphoma - 6.0 (exposed more than 20 days/yr) Non-Hodgkin's lymphoma - 8.0 (frequent users of herbicides)	Lack of substantiation of exposure. Data are based on questionnaire responses and, as such, recall bias may be present. Substances used other than 2,4-D were not identified on product.

(continued on the following page)

TABLE 6. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Woods et al. (1987)	Occupational exposure to phenoxy herbicides or chlorophenols based on personal interviews.	Significant elevated risks of NHL were observed in subgroups thought to have possibly heavier exposures to herbicides. An excess borderline significant risk of chloracne was found in workers reporting NHL.	Lymphomas 576 NHL 694 referents Risk was 1.33 ($p<0.05$) farmers Risk was 4.8 ($p<0.05$) forestry herbicide applicators Risk was 1.71 ($p<0.05$) potential exposure to phenoxy herbicides for 15+ years during period prior to 15 years before cancer registration. Risk was 2.12, presence of chloracne ($p<0.075$)	Possibly confounding exposure to organochlorine insecticides and organic solvents. Possible misclassification of exposure because of use of questionnaire to elicit past history of exposure.

TABLE 7. PROPORTIONATE MORTALITY STUDIES OF POLYCHLORINATED DIBENZO-p-DIOXINS, PARTICULARLY 2,3,7,8-TCDD

Author(s)	Exposure	Salient points	Major findings	Major concerns
Milham (1982)	Phenoxy herbicides (chiefly 2,4-D) and chlorophenols used in agriculture, forestry, and wood products in Washington State. Source of data not stated.	A significant excess of STS in farmers. A significant excess of Hodgkin's disease in paper/pulp millworkers and higher risk of non-Hodgkin's lymphoma in paper/pulp millworkers.	STS (49 cases) Hodgkin's disease (109 cases) Non-Hodgkin's lymphoma (188 cases) Risks STS = 1.52 ($p<0.05$) PMR Hodgkin's disease = 1.94 ($p<0.05$) PMR Non-Hodgkin's lymphoma = 1.33 PMR	There is no evidence of actual exposure to the phenoxy herbicides and/or chlorophenols cited by the authors. Proportionate mortality rates have inherent weaknesses in that site-specific findings are not independent of each other. An increase (or decrease) in the risk of one will depress (or increase artificially) the risk of all others.

(continued on the following page)

TABLE 7. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Kogan and Clapp (1985)	Service in Vietnam. Cohort was provided by Massachusetts Office of Veterans Services. Referent group was non-Vietnam veterans or Massachusetts white males.	Deaths due to stroke, kidney, and connective tissue cancer were significantly elevated among Massachusetts Vietnam veterans compared to non-Vietnam veterans.	Risk of connective tissue cancer (9 cases) = 4.73 ($p<0.01$) SPMR. Risk of connective tissue cancer SMOR = 5.16 ($p<0.01$) non-Vietnam veterans; 5.87 ($p<0.01$) Massachusetts white males. Risk of kidney cancer = 3.53 ($p<0.01$) SPMR.	There is no evidence of actual exposure to the phenoxy herbicides (Agent Orange) used in Vietnam. Proportionate mortality rates have inherent weaknesses in that site-specific cancer is not independent of other cancers in the analysis. An increase in one cancer will produce an overall decrease in all the others.

TABLE 7. (continued)

Author(s)	Exposure	Salient points	Major findings	Major concerns
Kogan and Clapp (1985)	Service in Vietnam. Cohort was provided by Massachusetts Office of Veterans Services. Referent group was non-Vietnam veterans or Massachusetts white males.	Deaths due to stroke, kidney, and connective tissue cancer were significantly elevated among Massachusetts Vietnam veterans compared to non-Vietnam veterans.	Risk of connective tissue cancer (9 cases) = 4.73 ($p<0.01$) SPMR. Risk of connective tissue cancer SMOR = 5.16 ($p<0.01$) non-Vietnam veterans; 5.87 ($p<0.01$) Massachusetts white males. Risk of kidney cancer = 3.53 ($p<0.01$) SPMR.	There is no evidence of actual exposure to the phenoxy herbicides (Agent Orange) used in Vietnam. Proportionate mortality rates have inherent weaknesses in that site-specific cancer is not independent of other cancers in the analysis. An increase in one cancer will produce an overall decrease in all the others.

REFERENCES

Axelson, O. (1986, Jan. 21) Rebuttals of the final report on cancer by the Royal Commission on the use and effects of chemical agents on Australian personnel in Vietnam. Department of Occupational Medicine, University Hospital, Universitetet Linkoping, Linkoping, Sweden.

Axelson, O.; Sundell, L.; Anderson, K.; Edling, C.; Hogstedt, C.; Kling, H. (1980) Herbicide exposure and tumor mortality: an updated epidemiologic investigation of Swedish railroad workers. Scand. J. Work Environ. Health 6:73-79.

Australian Royal Commission. (1985) Cancer, Vol. 4. In: Final report--Royal Commission on the use and effects of chemical agents on Australian personnel in Vietnam. Canberra, Australia: Australian Government Publishing Service, pp. VIII-166.

Balarajan, R.; Acheson, E.D. (1984) Soft tissue sarcomas in agriculture and forestry workers. J. Epidemiol. Community Health 38:113-116.

Bishop, C.M.; Jones, A.H. (1981) Non-Hodgkin's lymphoma of the scalp in workers exposed to dioxins. The Lancet 2(8242):369.

Buesching, D.P.; Wollstadt, L. (1984) Cancer mortality among farmers. J. Natl. Cancer Inst. 72(3):503.

Burmeister, L.F.; Everett, G.D.; Van Lier, S.F.; Isacson, P. (1983) Selected cancer mortality and farm practices in Iowa. Am. J. Epidemiol. 118(1): 72-77.

Cantor, K.P. (1982) Farming and mortality from non-Hodgkin's lymphoma: a case-control study. Int. J. Cancer 29:239-247.

Cook, R.R. (1981) Dioxin, chloracne and soft tissue sarcoma. The Lancet 1:618-619.

Cook, R.R.; Cartmill, J.B. (1984) Dioxin: comparing apples and oranges. Chem. Tech. :534-537.

Cook, R.R.; Bond. G.G.; Olsen, R.A.; Ott, M.G.; Gondek, M.R. (1986) Evaluation of the mortality experience of workers exposed to the chlorinated dioxins. Chemosphere 15(9-12):1769-1776.

Eriksson, M.; Hardell, L.; O;Berg, N.; Moller, T.; Axelson, O. (1979) Case-control study on malignant mesenchymal tumors of the soft tissue and exposure to chemical substances. Lakartidningen 76:3872-3875. (translation)

Eriksson, M.; Hardell, L.; O'Berg, N.; Moller, T.; Axelson, O. (1981) Soft tissue sarcomas and exposure to chemical substances: a case-referent study. Br. J. Ind. Med. 38:27-33.

Fett, M.J.; Dunn, M.; Adena, M.A.; O'Toole, B.I.; Forcier, L. (1984) Australian veterans health studies: the mortality report. Part I: A retrospective cohort study of mortality among Australian national servicemen of the Vietnam conflict era, and an executive summary of the mortality report. Canberra, Australia: Australian Government Publishing Service.

Fingerhut, M.; Halperin, W.; Honchar, P.; Smith, A.B.; Groth, D.; Russell, W.O. (1984) An evaluation of reports of dioxin exposure and soft tissue sarcoma pathology in U.S. chemical workers. Scan. J. Work. Environ. Health 10:299-303.

Greenwald, P.; Kovasznay, B.; Collins, D.: Therriault, G. (1984) Sarcomas of soft tissues after Vietnam service. J. Natl. Cancer Inst. 73(5):1107-1109.

Hardell, L. (1981) Relation of soft tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scand. J. Work Environ. Health 7:119-130.

Hardell, L.; Eriksson, M. (1986) Soft tissue sarcoma and exposure to dioxins. The Lancet p. 868.

Hardell, L.; Sandstrom, A. (1979) Case-control study: soft tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br. J. Cancer 39:711-717.

Hardell, L., Eriksson, M.; Lenner, P.; Lundgren, E. (1981) Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br. J. Cancer 43:169-176.

Hoar, S.K.; Blair, A.; Holmes, F.F.; Boysen, C.D.; Robel, R.J.; Hoover, R.; Fraumeni, J.F., Jr. (1986) Agricultural herbicide use and risk of lymphoma and soft tissue sarcoma. J. Am. Med. Assoc. 256(9):1141-1147.

Hueper, W.C.; Conway, W.D. (1964) Chemical carcinogenesis and cancers. Springfield, MA: C.A. Thomas.

Johnson, F.E.; Kugler, M.A.; Brown, S.M. (1981) Soft tissue sarcomas and chlorinated phenols. The Lancet 2(8236):40.

Kang, H.K.; Weatherbee, L.; Breslin, P.P.; Lee, Y.; Shepard, B.M. (1986) Soft tissue sarcomas and military service in Vietnam: a case comparison group analysis of hospital patients. J. Occup. Med. 28(12):1215-1218.

Kang, H.K.; Enziger, F.; Breslin, P.; Feil, M.; Lee, Y.; Shepard, B. (1987) Soft tissue sarcoma and military service in vietnam: a case-control study. J. Natl. Cancer Inst. 79(4)693-699.

Karon, J.M.; Flanders, W.D.; Devine, O.J.; Needham, L.L.; Patterson, D.G. Jr. (1987) Serum 2,3,7,8-tetrachlorodibenzo-p-dioxin levels in U.S. Army Vietnam and non-Vietnam veterans. Presented at the 7th International Symposium on Chlorinated Dioxins and Related Compounds; Oct. 4-9; Univ. of Nevada, Las Vegas, NV.

Kogan, M.D.; Clapp, R.W. (1985) Mortality among Vietnam veterans in Massachusetts, 1972-1983. Massachusetts Office of the Commissioner of Veterans Services, Agent Orange Program, Massachusetts Department of Public, Division of Health Statistics and Research, Boston, MA.

Lathrop, G.D.; Wolfe, W.H.; Albanese, R.A.; Moynahan, P.M. (1984a) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides: baseline morbidity study results. USAF School of Aerospace Medicine, Brooks Air Force Base, Texas. 19 chapters.

Lathrop, G.D.; Wolfe, W.H.; Albanese, R.A.; Moynahan, P.M. (1984b) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides and associated dioxins. Presented at the Fourth International Symposium on Chlorinated Dioxins and Related Compounds; October 16-18; Ottawa, Ontario (abstract).

Lynge, E. (1985) A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. Br. J. Cancer 52:259-270.

May, G. (1982) Tetrachlorodibenzodioxin: a survey of subjects ten years after exposure. Br. J. Ind. Med. 39(2):128-135.

Merlo, F.; Puntoni, R. (1986) Soft tissue sarcomas, malignant lymphomas, and 2,3,7,8-TCDD exposure in Seveso. The Lancet p. 1455.

Michigan Department of Public Health. (1983a, May 4) Evaluation of congenital malformation rates for midland and other selected Michigan counties compared nationally and statewide 1970-1981. Michigan Department of Public Health.

Michigan Department of Public Health. (1983b, May 4) Evaluation of soft and connective tissue cancer mortality rates for Midland and other selected Michigan counties compared nationally and statewide. Michigan Department of Public Health.

Milham, S., Jr. (1982) Herbicides, occupation, and cancer. The Lancet pp. 1464-1465.

Moses, M.; Selikoff, I.J. (1981) Soft tissue sarcomas, phenoxy herbicides and chlorinated phenols. The Lancet 1:(8234):1370.

Olsson, H.; Brandt, L. (1981) Non-Hodgkin's lymphoma of the skin and occupational exposure to herbicides. The Lancet p. 579.

Ott, M.G.; Olson, R.A.; Cook, R.R.; Bond, G.G. (1987) Cohort mortality study of chemical workers with potential exposure to the higher chlorinated dioxins. J. Occup. Med. 29(5):422-429.

Pazderova-Vejlupkova, J.; Nemcova, M.; Pickova, J.; Jirasek, L.; Lukas, E. (1981) The development and prognosis of chronic intoxication by tetrachlorodibenzo-p-dioxin in men. Arch. Environ. Health 36(1):5-11.

Pirkle, J.L.; Wolff, W.H.; Patterson, D.G.; Needham, L.L.; Michael, J.E.; Miner, J.C.; Peterson, M.R. (1987) Estimates of the half-life of 2,3,7,8-TCDD in ranch hand veterans. Presented at the 7th International Symposium on Chlorinated Dioxins and Related Compounds; Oct. 4-9; Univ. of Nevada, Las Vegas, NV.

Puntoni, R.; Merlo, F.; Fini, A.; Meazza, L.; Santi, L. (1986) Soft tissue sarcomas in Seveso. The Lancet p. 525.

Riihimaki, V.; Sisko, A.; Hernberg, S. (1982) Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland. Scand. J. Work Environ. Health 8:37-42.

Riihimaki, V.; Asp, S.; Pukkala, E.; Hernberg, S. (1983) Mortality and cancer morbidity among chlorinated phenoxy acid applicators in Finland. Chemosphere 12(4/5):779-784.

Sarma, P.R.; Jacobs, J. (1981) Thoracic soft tissue sarcoma in Vietnam veterans exposed to Agent Orange. The Lancet 306(18):1109.

Smith, A.H.; Pearce, N.E. (1986) Update on soft tissue sarcoma and phenoxyherbicides in New Zealand. Chemosphere 15(9-12):1795-1798.

Smith, A.H.; Waxweiler, R.J.; Tyroler, H.A. (1980) Epidemiologic investigation of occupational carcinogenesis using a serially additive expected dose model. Am. J. Epidemiol. 112:787-797.

Smith, A.H.; Pearce, N.E.; Fisher, D.O.; Giles, H.J.; Teague, C.A.; Howard, J.K. (1984) Soft tissue sarcoma and exposure to phenoxyherbicides and chlorophenols in New Zealand. J. Natl. Cancer Inst. 73(5):1111-1117.

Thiess, A.M.; Frentzel-Beyme, R.; Link, R. (1982) Mortality study of persons exposed to dioxin in a trichlorophenol-process accident that occurred in the BASF AG on November 17, 1953. Am. J. Ind. Med. 3:179-189.

U.S. Environmental Protection Agency (1985). Health assessment document for polychlorinated dibenzo-p-dioxins. Office of Health and Environmental Assessment. Washington, DC. EPA/600/8-84/014F. NTIS PB86-122546/AS.

Wiklund, K.; Holm, L.E. (1986) Soft tissue sarcoma risk in Swedish agricultural and forestry workers. J. Natl. Cancer Inst. 76(2):229-234.

Wiklund, K.; Dich, J.; Holm, L.E. (1987) Risk of malignant lymphoma in Swedish pesticide appliers. Br. J. Cancer 56:505-508.

Wolfe, W.H.; Michalek, U.E.; Albanese, R.A.; Lathrop, G.D.; Moynahan, P.M. (1984) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides: mortality update, 1984. USAF School of Aerospace Medicine, Brooks Air Force Base, Texas.

Wolfe, W.H.; Lathrop, G.D.; Albanese, R.A.; Moynahan, P.M. (1985) An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides and associated dioxins. Chemosphere 14(6/7):707-716.

Woods, J.S.; Polissar, L.; Severson, R.K.; Heuser, L.S.; Kulander, B.G. (1987) Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in Western Washington. J. Natl. Cancer Inst. 78(5):899-910.

Young I.L.; Racy, C.L.; Asire, A.J., eds. (1982) Surveillance, epidemiology and end results: incidence and mortality data, 1973-77. Natl. Cancer Inst. Monogr. 57:14, 32, 72, 84.

Zack, J.A.; Suskind, R.R. (1980) The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J. Occup. Med. 22(1):11-14.

February 1988
Review Draft

APPENDIX C

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF 2,3,7,8-TCDD

G. L. Kimmel, Ph.D.
Reproductive Effects Assessment Group

Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

EXECUTIVE SUMMARY

There is not sufficient evidence to link 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) to human reproductive or developmental toxicity; however, it has been shown to be a reproductive and developmental toxicant in animal studies. Among the effects that have been reported are reduced fertility, litter size, postnatal survival, and offspring body weight, as well as an increase in structural malformations. Effects on the male and female gonads and on the female menstrual/estrous cycle have also been reported. Reproductive and developmental effects have been observed in a variety of species, indicating that the toxicity is not a species-specific event.

The studies on reproductive function and fertility remain the basis for establishing the lowest effective (toxic) exposure level. It appears that a 0.01 ug/kg/day exposure is the lowest effect level that can be supported by the data, although further analysis of the data may provide some support for a lower effect level of 0.001 ug/kg/day. In addition, a detailed analysis of studies in the subhuman primate may also provide support for a lower effect level of 0.001 ug/kg/day. In relation to developmental toxicity, a large number of studies in a variety of species has demonstrated that 2,3,7,8-TCDD is a developmental toxicant. Collectively, these studies indicate that long-term, low-dose exposure is of concern relative to the potential for altering reproductive function and fertility. The results also demonstrate that acute and short-term exposures are effective in causing altered development, and therefore, should also be of concern.

INTRODUCTION

2,3,7,8-TCDD has been shown to be a reproductive and developmental toxicant in animal studies. Among the effects that have been reported are reduced fertility, litter size, postnatal survival, and offspring body weight, as well as an increase in structural malformations. 2,3,7,8-TCDD also affects the male and female reproductive systems. Gonadal dysfunction has been demonstrated in both sexes, and alterations of normal reproductive cycles have been reported in the female. Although there have been accidental exposures of humans to mixtures containing 2,3,7,8-TCDD, there is not sufficient evidence from the case reports and epidemiologic studies that have been carried out to date to link 2,3,7,8-TCDD to human reproductive or developmental toxicity (U.S. EPA, 1986a). Much of the information on human exposure is covered in Appendix D.

In line with the original request for the development of this review, this appendix covers the effects of 2,3,7,8-TCDD on the integrity of the reproductive system and fertility and on prenatal and early postnatal development. The appendix is not inclusive of all studies on these effects. Rather, it focuses on the key studies and issues that may go into an overall risk characterization. The present reviewer has tried to present a balanced view of the studies and the uncertainties inherent in the data and the analysis. Studies on other congeners of polychlorinated dibenzo-p-dioxins or of 2,3,7,8-TCDD as a mixture or contaminant of other agents are not included, except as support where appropriate. A more comprehensive review of 2,3,7,8-TCDD's toxicity and its relation to risk characterization can be found in the Health Assessment Document (HAD) for Polychlorinated Dibenzo-p-Dioxins

(U.S. EPA, 1985).

ANIMAL STUDIES

REPRODUCTIVE FUNCTION/FERTILITY

The study by Murray et al. (1979) continues to be the guidepost for setting standards of exposure relative to reproduction. The study employs a multigeneration approach and examines the exposure of male and female rats over three generations to relatively low levels of 2,3,7,8-TCDD (0, 0.001, 0.01, and 0.1 ug 2,3,7,8-TCDD/kg body weight/day). The analysis of the data is made somewhat difficult by considerable variation in the fertility index in both control and exposed groups. In addition, the number of impregnated animals in the exposed groups was lower than desirable (Palmer, 1981). However, there were effects that cannot be automatically associated with the variation in the fertility index, including an increased time between first cohabitation and delivery, a decrease in litter size, a decrease in the gestational survival index, and a decrease in postnatal body weight. Specifically, Murray et al. reported statistically significant changes in several of the measured parameters, and these are outlined in Table 1.

While there is no dispute over the reproductive toxicity seen in this study, there is some disagreement over the appropriate effect levels. Murray et al. (1979) indicated that the lowest statistically significant adverse effect was observed at 0.01 ug/kg/day and that a no-effect level could be established at 0.001 ug/kg/day. In a reanalysis of this study, however, Nisbet and Paxton (1982) argued that the analysis of Murray et al. (1979) was limited by the statistical approach used. Nisbet and Paxton applied a different

TABLE 1. SUMMARY OF EFFECTS OF 2,3,7,8-TCDD ON REPRODUCTION

Parameter	Generation	ug TCDD/kg/day[a]		
		0.001	0.001	0.1
Fertility	f_0	---	---	dec
	f_0	---	---	dec
	f_1	---	dec	
	f_2	---	dec	
Litter size	f_{1a}	---	---	dec
	f_{1b}	---	---	dec
	f_2	---	dec	
	f_3	---	dec	
Gestation Survival	f_{1a}	---	---	dec[b]
	f_{1b}	---	---	---[c]
	f_2	dec	dec	
	f_3	---	dec	
Postnatal survival	f_{1a}	dec	dec	[b]
	f_{1b}	inc	---	---[c]
	f_2	---	dec	
	f_3	---	---	
Postnatal body weight	f_{1a}	---	---	[b]
	f_{1b}	---	---	---[c]
	f_2	---	dec	
	f_3	---	dec	

[a] (---), unaffected; dec, decreased; inc, increased.

[b] No liveborn offspring.

[c] One litter only.

SOURCE: Murray et al., 1979.

statistical approach which included the pooling of data across all generations, and their reanalysis indicated that 0.001 ug/kg/day (the lowest dose used) was an effect level and that a no-effect level could not be set. The authors of the HAD for Polychlorinated Dibenzo-p-Dioxins (U.S. EPA, 1985) accepted this argument and used the Nisbet and Paxton reanalysis to establish a lowest observed adverse effect level (LOAEL) of 0.001 ug/kg/day. However, the HAD (U.S. EPA, 1985) also noted that the FIFRA Scientific Advisory Panel (SAP) did not feel that the effects were consistent enough at 0.001 ug/kg/day and that this would have to be considered a no observed effect level (NOEL). Since this latter decision by the SAP was made before the Nisbet and Paxton reanalysis, it is not possible to know if the decision would have been different in light of the reanalysis. However, the present reviewer feels that effect levels should not be set on the basis of the Nisbet and Paxton reanalysis. While it appears that Nisbet and Paxton's approach for increasing the limited statistical power of the Murray et al. (1979) study is appropriate statistically, it is difficult to see the biological rationale for pooling the data. Litters from different generations (or from subsequent matings within a generation) are not the same. They have different histories of exposure and each is tied to the effect of the agent on its parental generation. Thus, as a general rule, pooling of data from different generations would seem biologically inappropriate. Unless some specific exception can be identified, it is not clear how pooling can be biologically justified in this case.

A limited review of a report by Murray et al. (1978), which served as Exhibit 77 in a 1980 EPA hearing, has raised some questions relative to the offspring survival. The Murray et al. (1979) paper included the standard parameters of the Gestation Survival Index and Postnatal Survival Index as

measures of offspring survival (Table 1). These parameters showed significant changes, but not always in a dose-related fashion. This may be because offspring viability is examined during discrete periods of offspring development and the investigators do not report viability over the entire early postnatal period. Additional data from the 1978 report by Murray et al. has been summarily reviewed on the basis of overall offspring survival, i.e., not separating the Gestation Survival Index and Postnatal Survival Index. There appears to be a general pattern of decreased survival even at 0.001 ug/kg/day, if one assumes a survival rate of control offspring of 90%. Appropriate analytical techniques would have to be applied to confirm this. Two points should be raised regarding the data. The first is that the number of offspring used in the calculations of Murray et al. (1978) varied considerably among the control and two exposure groups. How this could affect the parameters is not entirely clear to this reviewer, but Bailar (1981) spoke to similar issues in his testimony and noted that he found the data suggestive of an effect at the 0.001 ug/kg/day level. The second point is the survival of the control population of offspring. In both the f_{1b} and the f_3 litters, survival of the controls by postnatal day 21 ranged from 70% to 80%. Although viability varies within any laboratory animal population, this figure seems low and may account for there not being an established decrease in offspring viability in these two groups at 0.001 ug/kg/day. A more detailed analysis of this data base may provide a clearer indication of the potential for decreased postnatal survival at the 0.001 level.

In addition to the data on offspring survival, the Murray et al. (1978) report summarizes their observations on renal pathology. When all observed effects on the kidney (i.e., "slightly dilated" and "dilated") are combined,

there appears to be an increase at the 0.001 and 0.01 ug/kg/day in the f_{1a} and f_{1b} litters. As has been pointed out, it is not entirely appropriate to combine these two end points, since slight dilation may be due to delayed development which may be transient in nature. Nevertheless, the kidney is a recognized end organ for 2,3,7,8-TCDD effects, and the findings of Moore et al. (1973) in the mouse indicate that the continuous exposure that is found in a three-generation study may be more likely to lead to the most obvious effects on kidney development. Research in this particular area is continuing. Abbott et al. (1987a, b) recently reported that the kidney alterations that occur in the mouse following a single, prenatal 12 ug/kg dose of 2,3,7,8-TCDD are consistent with true hydronephrosis.

Allen and his colleagues examined 2,3,7,8-TCDD effects on reproduction in the monkey (Allen et al., 1977; Allen et al., 1979; Barsotti et al., 1979; Schantz et al., 1979). In a series of studies, female rhesus monkeys were fed 50 or 500 ppt 2,3,7,8-TCDD for up to 9 months. Menstrual cycles and serum steroid levels were examined. Following 7 months of exposure, the females were bred. Females exposed to 500 ppt showed obvious clinical signs of 2,3,7,8-TCDD toxicity and lost weight throughout the study. Five of the eight monkeys died within one year after exposure was initiated. In a summary of the reproductive function and fertility of these animals, Allen et al. (1979) reported that although the menstrual cycle and menstruation were normal, there was a decrease in serum estradiol and progesterone in five of eight monkeys. Only three of the animals conceived, and only one was able to carry the pregnancy to term. Females exposed to 50 ppt 2,3,7,8-TCDD in the diet (Schantz et al., 1979) showed normal menstrual cycles and serum estradiol and progesterone through 6 months of exposure. When they were bred at 7 months, four of eight females did

not conceive and two of four that did could not carry the pregnancies to term. Only two conceptions resulted in normal births.

The results of this series of studies could potentially support a lower LOAEL than that reported by Murray et al. (1979) in the rat (i.e., 0.01 ug/kg/day). The high dose (500 ppt) resulted in considerable maternal toxicity and reproductive dysfunction, while a comparable exposure level (0.01 ug/kg/day) in the rat did not produce any significant clinical signs in the parental generation. This could indicate that the rhesus monkey is more sensitive to 2,3,7,8-TCDD when exposure occurs over long periods and when reproductive parameters are the critical end points. The low dose (50 ppt) was reported as resulting in specific reproductive dysfunction in the absence of maternal toxicity. Since this exposure level is calculated to be approximately 0.002 ug/kg/day, the report suggests that even lower doses are required for effects on reproductive function than are required in the rat and would support a lower adverse effect level. Unfortunately, much of the data on the monkeys has been presented in abstract form or as part of a review, and consequently, a critical analysis of the data is impossible. There has been some indication that studies of even lower levels (i.e., 5 and 25 ppt) showed signs of reproductive toxicity, and the data are now beginning to appear in the literature. Schantz et al. (1986) reported altered maternal care of offspring in monkeys exposed to 5 and 25 ppt for 45 to 49 months, and Bowman et al. (1987a, b) reported on altered maternal-infant interaction and other reproductive parameters at the Seventh International Symposium on Chlorinated Dioxins and Related Compounds. These reports are now being evaluated and, if supported, could significantly affect the adverse effect levels calculated for reproductive and developmental toxicity.

It is important to note that none of these findings establish an unequivocal effect at the 0.001 ug/kg/day or below level. However, the evidence is suggestive enough and the uncertainties are great enough that it would seem prudent to consider the 0.001 level as highly suspect.

DEVELOPMENT

Numerous studies have been done on the developmental toxicity of 2,3,7,8-TCDD, many of which have been summarized in the HAD for Polychlorinated Dibenzo-p-Dioxins (U.S. EPA, 1985). Of particular interest to this review are those studies which present data that may factor into an overall risk characterization: Courtney and Moore (1971), Giavini et al. (1982a), Khera and Ruddick (1973), McNulty (1980), Moore et al. (1973), Smith et al. (1976), and Sparschu et al. (1971).

Developmental toxicity following exposure to 2,3,7,8-TCDD has been demonstrated in different species, including the chicken, mouse, rat, rabbit, ferret, and monkey. Thus, developmental toxicity of 2,3,7,8-TCDD does not appear to be related to a species-specific metabolic or physiological response to exposure. While specific responses and effective doses do vary among species and among strains within a species (Courtney and Moore, 1971; Poland and Glover, 1980), developmental toxicity in response to 2,3,7,8-TCDD exposure can be expected to occur in all species.

The exposure range at which developmental toxicity first becomes apparent is 0.125 to 1.0 ug/kg/day when exposure occurs over a major period of organogenesis. The no-effect level appears to be approximately 0.1 ug/kg/day in rats, mice, and rabbits. Giavini et al. (1982a) did note an increase in extra ribs at 0.1 ug/kg/day in the rabbit. However, the number of ribs

normally varies between 12 and 13 in rabbits, and it would require a more detailed analysis of the data to establish this as a true effect level. Several laboratories have examined the effects of more acute exposures during organogenesis. Moore et al. (1973) demonstrated that a single oral dose of 1 ug/kg given to mice on gestation day 10 produced hydronephrosis. In an interesting extension of this finding, they investigated the postnatal development of the kidney in cross-fostered offspring following prenatal exposure. The frequency of hydronephrosis seen postnatally was largely dependent on whether the offspring were nursed by a dam that had also been prenatally exposed to 2,3,7,8-TCDD. Thus, it appeared that continued exposure during the lactation period was required to produce or maintain the greatest effect on kidney development during the postnatal period studied.

Except for the multigeneration studies, which tend not to critically evaluate many developmental end points, few studies have been carried out on developmental periods other than the period of organogenesis. Giavini et al. (1982b) did examine 2,3,7,8-TCDD exposure on gestation days 1-3 in the rat and reported possible delays in implantation and some effect on fetal weight and the kidney. There appear to be no studies on exposure during late prenatal development. As noted above, Moore et al. (1973) examined effects on the kidney postnatally following prenatal exposure of the dams, and demonstrated that transfer in the milk is a likely contributing factor to the developmental toxicity of 2,3,7,8-TCDD. There have also been reports describing the effects of 2,3,7,8-TCDD exposure on the developing immune system (see Appendix E). However, carefully designed studies on postnatal exposures or on changes in postnatal function following prenatal exposure have generally not been carried out, making it impossible to evaluate the potential effect of 2,3,7,8-TCDD on

the developing young animal.

In summary, studies on the developmental toxicity of 2,3,7,8-TCDD have clearly demonstrated that it is a developmental toxicant in a wide variety of species at relatively low doses. These studies have also shown that extended periods of exposure are not necessary for developmental toxicity to result. Thus, there is a need for concern over acute or short-term exposure to 2,3,7,8-TCDD. A composite review and summary of the studies on the developmental toxicity of 2,3,7,8-TCDD is limited by factors such as the simultaneous occurrence of maternal toxicity, the use of different animal strains and exposure routes, and in many studies the small number of animals per treatment group that are included in the final data analysis. In addition, there are differences in study designs and approaches to data analysis which must be considered in comparing the studies. These factors do not alter the finding that 2,3,7,8-TCDD is a developmental toxicant at very low exposure levels. However, they can potentially affect the final assessment of exposure levels that can be considered toxic.

MALE AND FEMALE REPRODUCTIVE SYSTEM

Specific components of the male and female reproductive systems are affected by 2,3,7,8-TCDD exposure. In the male, exposures above 1 ug/kg/day resulted in evidence of testicular atrophy with destruction of the seminiferous tubules and spermatogenic cells (Kociba et al., 1976; Norback and Allen, 1973; McConnell et al., 1978). However, following exposures of 0.001 to 0.1 ug/kg/day over a 2-year period, Kociba et al. (1978) reported that the male reproductive organs appeared to be unaffected, relative to the controls. In a study of offspring of male mice exposed to 0.16 to 2.4 ug/kg/day of 2,3,7,8-

TCDD combined with higher doses of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, there did not appear to be any effect on fetal or neonatal development or viability (Lamb et al., 1981). In the female, exposure to 1 to 2 ug/kg/day for 13 weeks resulted in changes in estrous cycles, anovulation, and signs of ovarian dysfunction (Kociba et al., 1976). At exposures of 0.001 to 0.01 ug/kg/day in a 2-year study, Kociba et al. (1978) reported no effects on the female reproductive system. At 0.1 ug/kg/day, a decrease in uterine changes such as endometrial hyperplasia were reported. As noted previously in the Allen et al. studies, female monkeys exposed to 500 ppt 2,3,7,8-TCDD were reported to exhibit changes in their serum steroid levels.

SUMMARY

2,3,7,8-TCDD has been shown to be a reproductive and developmental toxicant in animal studies. Among the effects that have been reported are reduced fertility, litter size, postnatal survival, and offspring body weight, as well as an increase in structural malformations. Effects on the male and female gonads and on the female menstrual/estrous cycle have also been reported. The effects have been observed in a variety of species, indicating that the toxicity is not a species-specific event and can be expected to occur in all species, including the human.

The studies on reproductive function and fertility remain the basis for establishing the lowest effective (toxic) exposure level. There is some disagreement, centered on the appropriate approach for data analysis, over the effect level based on the Murray et al. (1979) study. Based on the current information from this study, a 0.01 ug/kg/day level is the lowest effect level

that can be supported by the data. However, there is enough suggestive evidence to indicate a real potential for an effect at 0.001 ug/kg/day. Further analysis of this study and of the subhuman primate studies by Allen and his colleagues may provide support for a lower effect level of at least 0.001 ug/kg/day.

In relation to developmental toxicity, a large number of studies in a variety of species demonstrated that 2,3,7,8-TCDD is a developmental toxicant. Although a longer term exposure appears to cause effects at slightly lower doses, acute and short-term exposures are effective in causing altered development, and therefore, should also be of concern. When exposure occurs during the prenatal period of organogenesis, the lowest effect level is in the range of 0.125 to 1.0 ug/kg/day and the no-effect level is approximately 0.1 ug/kg/day. Studies focusing on other periods of development are limited, but they do indicate that exposure at any time during prenatal and early postnatal life must be considered a potential threat to normal development.

2,3,7,8-TCDD also affects the male and female reproductive systems. Unfortunately, the amount of attention that has been given to these areas of investigation has not been as great as that given to reproductive function and development. Gonadal dysfunction has been demonstrated in both sexes, and alterations of normal reproductive cycles have been demonstrated in the female. Continuing investigations of the effect of 2,3,7,8-TCDD and related agents on reproductive physiology and cellular events should increase our understanding of the potential effect of 2,3,7,8-TCDD on the male and female reproductive systems.

The uncertainties that arise from this data base are many, largely because the area of reproductive and developmental toxicology covers a wide range of

potential exposure-response scenarios, and because the reproductive and developing systems present constantly changing targets with which a toxicant may interact. In the case of 2,3,7,8-TCDD exposure, many of these uncertainties have been discussed in this review and should be taken into account in carrying out the final risk characterization. The laboratory data establishes 0.01 ug/kg/day as a lowest observed adverse effect level (LOAEL) when exposure is chronic. Standard approaches for applying uncertainty/ modifying factors and determining a reference dose (RfD) have been used (U.S. EPA, 1986b; 1987). An unequivocal no observed adverse effect level (NOAEL) can be questioned. A 1000-fold uncertainty factor (UF) can be applied to account for variation in sensitivity within the human population, uncertainty in extrapolating animal data to the human situation, and the use of a LOAEL instead of a NOAEL in calculating the reference dose. An additional modifying factor seems unnecessary, since the uncertainty factor accounts for many of the concerns of this reviewer, i.e., the true LOAEL/NOAEL and the potential for the pregnant woman and her offspring to be more sensitive than the average healthy adult. An exception to this position would arise if an actual effect level much below 0.001 ug/kg/day was established, and as noted above, suggestive evidence is accumulating that would support a lower NOAEL/LOAEL. The calculation of a reference dose (RfD) for reproductive and developmental toxicity, based on the current data and literature base, is as follows:

$$\begin{aligned} RfD &= LOAEL/UF \\ &= (0.01 \text{ ug/kg/day})/1000 \\ &= 1 \times 10^{-5} \text{ ug/kg day} \end{aligned}$$

This does not account for potential or actual human exposures which would have to be factored into the final risk characterization.

Future investigations should be encouraged to more clearly define the substantial data base that already exists. In the area of reproductive function and fertility, it would be helpful if a more critical review of the data of the three-generation rat study and the monkey studies was carried out to determine if a lower effective dose can be established. Additionally, a carefully designed multigeneration study could address some of the limitations of previous studies and fill certain data gaps. However, multigeneration studies are not easily designed, executed, or evaluated, and this step should only be taken when it is obvious that these data are necessary. In the area of developmental toxicology, the most pressing needs seem to be the evaluation of toxicity during periods of development that have not been adequately assessed, i.e., the late prenatal and early postnatal periods. With regard to the postnatal period, the potential for childhood exposure from breast feeding and from other environmental sources (e.g., ingestion of soil) seems considerable, and it has been suggested that the child may be particularly sensitive to exposure to polychlorinated dibenzo-p-dioxins. As relates to reproductive and developmental toxicity in general, a greater effort should be directed at identifying the effects of polychlorinated dibenzo-p-dioxins on hormonal regulation and normal cellular and tissue functions. There is evidence that 2,3,7,8-TCDD influences steroid metabolism and may be associated with steroid action at the cellular receptor level. Pratt and his colleagues (Dencker and Pratt, 1981; Pratt et al., 1984) have also shown a correlation in various mouse strains between the susceptibility to induction of cleft palate and the occurrence of the 2,3,7,8-TCDD receptor, and have proposed that 2,3,7,8-TCDD

exerts its teratogenic effect on the palate directly through this receptor. A considerable amount of literature has developed on the mechanisms of cellular interaction and action aspects of 2,3,7,8-TCDD, as well as on the structure-activity relationships of 2,3,7,8-TCDD with other dioxins and related compounds (recent reviews include: Safe, 1986; Silbergeld and Mattison, 1987). Efforts should be made to incorporate this information and to evaluate 2,3,7,8-TCDD within the context of the larger family of related agents. As additional information becomes available, this review will be updated and reconsidered relative to its appropriateness in defining critical studies and issues related to the reproductive and developmental toxicity of 2,3,7,8-TCDD.

REFERENCES

Abbott, B.D.; Birnbaum, L.S.; Pratt, R.M. (1987a) TCDD produces hydronephrosis in fetal mice by inducing hyperplasia of the ureteric epithelium. The Toxicologist 7:124. Abstract 496.

Abbott, B.D.; Birnbaum, L.S.; Pratt, R.M. (1987b) TCDD effects the basal lamina and extracellular matrix of the fetal mouse kidney. The Toxicologist 7:124, Abstract 495.

Allen, J.R.; Barsotti, D.A.; Lambrecht, L.K.; Van Miller, J.P. (1979) Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann. NY Acad. Sci. 320:419-425.

Allen, J.R.; Barsotti, D.A.; Van Miller, J.P.; Abrahamson, L.J.; Lalich, J.J. (1977) Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food Cosmet. Toxicol. 15:401-410.

Bailar, J.C., III. (1981) Direct testimony of Dr. John C. Bailar, III. FIFRA Docket No. 415, et al., Exhibit 936. U.S. Environmental Protection Agency, Washington, DC.

Barsotti, D.A.; Abrahamson, L.J.; Allen, J.R. (1979) Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. Bull. Environ. Contam. Toxicol. 21:463-469.

Bowman, R.E.; Schantz, S.L.; Gross, M.L.; Ferguson, S. (1987a) Behavioral effects in monkeys exposed to 2,3,7,8-TCDD transmitted maternally during gestation and for four months of nursing. Presented at the Seventh International Symposium on Chlorinated Dioxins and Related Compounds, Las Vegas, Nevada. Abstract AT02.

Bowman, R.E.; Schantz, S.L.; Weerasinghe, N.C.A.; Gross, M.L.; Barsotti, D.A. (1987b) Chronic dietary intake of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at 5 and 25 parts per trillion in the monkey: TCDD kinetics and dose-effect estimate of reproductive toxicity. Presented at the Seventh International Symposium on Chlorinated Dioxins and Related Compounds, Las Vegas, Nevada. Abstract AT03.

Courtney, K.D.; Moore, J.A. (1971) Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 20:396-403.

Dencker, L.; Pratt, R.M. (1981) Association between the presence of the Ah receptor in embryonic murine tissues and sensitivity to TCDD-induced cleft palate. Teratogenesis Carcinog. Mutagen. 1:399-406.

Giavini, E.; Prai, M.; Vismara, C. (1982a) Rabbit teratology study with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Res. 27:74-78.

Giavini, E.; Prai, M.; Vismara, C. (1982b) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin administered to pregnant rats during the preimplantation period. Environ. Res. 29:185-189.

Khera, K.S.; Ruddick, J.A. (1973) Polychlorodibenzo-p-dioxins: perinatal effects and the dominant lethal test in Wistar rats. In: Blair, E.H., ed. Chlorodioxins-origin and fate. Adv. in Chemistry Series 120. Washington, DC.: American Chemical Society.

Kociba, R.J.; Keeler, P.A.; Park, C.N.; Gehring, P.J. (1976) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): results of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35:553-574.

Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; Carreon, R.M.; Wade, C.E.; Dittenber, D.A.; Kalnins, R.P.; Frauson, L.E.; Park, C.N.; Barnard, S.D.; Hummel, R.A.; Humiston, C.G. (1978) Results of a two-year chronic toxicity and oncongenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46:279-303.

Lamb, J.C., IV.; Moore, J.A.; Marks, T.A.; Haseman, J.K. (1981) Development and viability of offspring of male mice treated with chlorinated phenoxy acids and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Toxicol. Environ. Health 8:835-844.

McConnell, E.E.; Moore, J.A.; Haseman, J.K.; Harris, M.W. (1978) The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44:335-356.

McNulty, W.P. (1980) Direct testimony of Dr. Wilbur P. McNulty, FIFRA Docket Nos. 415 et al., Exhibit No. 106. U.S. Environmental Protection Agency, Washington, DC.

Moore, J.A.; Gupta, B.N.; Zinkl, J.G.; Vos, J.G. (1973) Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ. Health Perspect. 5:81-85.

Murray, F.J.; Smith, F.A.; Nitschke, K.D.; Humiston, C.G.; Kociba, R.J.; Schwetz, B.A. (1979) Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50:241-252.

Murray, F.J.; Smith, F.A.; Nitschke, K.D.; Humiston, C.G.; Kociba, R.J.; John, J.A.; Blogg, C.D.; Schwetz, B.A. (1978) Three-generation study of rats ingesting 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Report of the Dow Chemical Company, Exhibit 77, FIFRA Docket Nos. 415 et al. U.S. Environmental Protection Agency, Washington, DC.

Nisbet, I.C.T.; Paxton, M.B. (1982) Statistical aspects of three-generation studies of the reproductive toxicity of 2,3,7,8-TCDD and 2,4,5-T. The American Statistician 36:290-298.

Norback, D.H.; Allen, J.R. (1973) Biological responses of the nonhuman primate, chicken, and rat to chlorinated dibenzo-p-dioxin ingestion. Environ. Health Perspect. 5:233-240.

Palmer, A.K. (1981) Regulatory requirements for reproductive toxicology: theory and practice. In: Kimmel, C.; Buelke-Sam, J., eds. Developmental Toxicology. New York: Raven Press.

Poland, A.; Glover, E. (1980) 2,3,7,8-tetrachlorodibenzo-p-dioxin: segregation of toxicity with the Ah locus. Mol. Pharmacol. 17:86-94.

Pratt, R.M.; Grove, R.I.; Kim, C.S.; Dencker, L.; Diewert, V.M. (1984) Mechanism of TCDD-induced cleft palate in the mouse. In: Poland, A.; Kimbrough, R.D., eds. Biological mechanisms of dioxin action. Banbury Report No. 18. Cold Spring Harbor Lab.

Safe, S.H. (1986) Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. Annu. Rev. Pharmacol. Toxicol. 26:371-399.

Schantz, S.L.; Barsotti, D.A.; Allen, J.R. (1979) Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 48:(2)A180.

Schantz, S.L.; Laughlin, N.K.; Van Valkenberg, H.C.; Bowman, R.E. (1986) Maternal care by rhesus monkeys of infant monkeys exposed to either lead or 2,3,7,8-tetrachlorodibenzo-p-dioxin. Neurotoxicology 7:637-650.

Silbergeld, E.K.; Mattison, D.R. (1987) Experimental and clinical studies on the reproductive toxicology of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Am. J. Ind. Med. 11:131-144.

Smith, F.A.; Schwetz, B.A.; Nitschke, K.D. (1976) Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 Mice. Toxicol. Appl. Pharmacol. 38:517-523.

Sparschu, G.L.; Dunn, F.L.; Rowe, V.K. (1971) Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Food Cosmet. Toxicol. 9:405-412.

U.S. Environmental Protection Agency (EPA) (1985) Health assessment document for polychlorinated dibenzo-p-dioxins. Office of Health and Environmental Assessment. EPA/600/8-84/014F. NTIS PB86-122546/AS.

U.S. Environmental Protection Agency (EPA) (1986a) Teratology and reproduction studies with TCDD. In: Pitot, H., ed. The report of the "dioxin" Update Committee. Office of Pesticides and Toxic Substances, Washington, DC.

U.S. Environmental Protection Agency (EPA) (1986b) Guidelines for the health assessment of suspect developmental toxicants. Federal Register 51:34028-34040.

U.S. Environmental Protection Agency (EPA) (1987) Reference dose (RfD): description and use in health risk assessments. Integrated Risk Information System (IRIS): Appendix A. Online. Intra-Agency Reference Dose Workgroup, Office of Health and Environmental Assessment. Environmental Criteria and Assessment Office, Cincinnati, OH. EPA/600/8-86/032a.

February 1988
Review Draft

APPENDIX D

EPIDEMIOLOGIC DATA ON REPRODUCTION AND EXPOSURE TO 2,3,7,8-TCDD: ITS USEFULNESS IN QUANTITATIVE RISK ASSESSMENT

Sherry G. Selevan, Ph.D.
Reproductive Effects Assessment Group

Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

INTRODUCTION

The following is a discussion of epidemiologic data on the reproductive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and their usefulness in qualitative and quantitative risk assessment. Several factors affect the usefulness of these studies. First, and probably most important, is the assessment of exposure. Other important factors include the biologic plausibility of the effects of exposure, given the time frames of exposure and its association with relevant outcomes, and the difficulty of attributing the effects of combined exposure to polychlorinated dibenzo-p-dioxins (hereafter referred to as "dioxin"), a contaminant in herbicides or pesticides.

Due to the narrow focus of this report, that is, the use of existing reproductive data in the risk assessment of dioxin, certain restrictions will be made on the data discussed: The usefulness of data for risk assessment depends upon the manner in which the probabilities of exposure for the study members are determined. The quality of exposure data may range from very indirect data to detailed industrial hygiene or environmental monitoring. These indirect data are less useful and result from assumptions that individuals were exposed due to his/her presence in a potentially exposed region/plant during a specific time period. More useful data would include measurement of actual levels of dioxin in air, soil, or water with the most useful data describing the individuals' levels of exposure. Studies with only indirect, assumed exposure data contribute little to a risk assessment because of limited confidence in the ability to determine whether a given individual was actually exposed. Misclassification of exposure will inevitably occur, typically resulting in a reduction of the estimate of risk (if such a risk

truly exists). Studies with more detailed exposure data will contribute more specific information to such a risk assessment.

ROUTES OF EXPOSURE

Humans may be exposed to 2,3,7,8-TCDD through several routes: dermal, ingestion, and inhalation. The rates of absorption through these routes vary, as do the important routes for each individual. The routes for each individual can be affected by such characteristics as location during spraying (e.g., Vietnam/Oregon forests), diet (e.g., consumption of contaminated water/fish), and work practices for those occupationally exposed. As these characteristics vary, so will each person's effective dose. Table 4 (U.S. EPA, 1988) describes the absorption fraction for dioxin from several routes: soil ingestion - 0.3, dermal exposure to soil - 0.005, vapor inhalation - 0.75, fat ingestion from dairy products or beef - 0.68, dust inhalation - 0.27, fish ingestion - 0.68, and surface water ingestion - 0.5. For example, an individual exposed to equal amounts of 2,3,7,8-TCDD from different routes may have radically different internal doses (e.g., during the ICMESA plant explosion in Seveso, Italy, an individual would have a different potential exposure than she/he would have if exposed to soil dust later on). Consequently, each individual's actual dose is difficult to estimate accurately in an epidemiologic study. Another potential source of 2,3,7,8-TCDD exposure of potential reproductive importance may be the infant's exposure through human breast milk (Rappe, 1985; Schecter et al., 1987; van den Berg et al., 1986).

The epidemiologic literature, while limited at this point in time, seems to cover two broad categories: In occupational settings, paternal effects have

been examined (e.g., aerial sprayers in Vietnam, Vietnam veterans, agricultural sprayers, and employees of manufacturers of 2,4,5-trichlorophenoxyacetic acid [2,4,5-T]). Birth defects and fetal loss have been examined through environmental exposures (e.g., aerial spraying of 2,4,5-T in Oregon, New Zealand, and Vietnam, and the ICMESA plant accident in Seveso, Italy). In these settings, separation of maternal and paternal effects is very difficult. The only exception may be in such settings as the plant accident in Seveso where the woman may already be pregnant and in utero exposures are the ones of importance. None of these exposures are "clean;" that is, of dioxin alone. All the human exposures to dioxin have been as a contaminant of some other agent (e.g., 2,4,5-T), and typically, only sketchy qualitative and quantitative exposure data were available. Therefore, it is difficult to separate out the effects of dioxin from the agent it contaminates. Only some very general conclusions may be drawn from the relative toxicities of the associated compounds.

A number of reviews have discussed the human data in detail (Kimbrough et al., 1984; Constable and Hatch, 1985; Friedman, 1984; Hatch, 1984; Hatch and Stein, 1986; U.S. EPA, 1985). These efforts will not be repeated here, but the discussion will be limited to several key areas of study and certain studies of special interest to help define the limits of our knowledge on the human reproductive effects of dioxin.

STUDIES OF VIETNAM VETERANS

Two studies have examined the reproductive effects of 2,3,7,8-TCDD and defoliants in U.S. Vietnam veterans. The primary exposure was to Agent Orange

which consists of defoliants 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T, the latter contaminated with 2,3,7,8-TCDD. (Agents Purple, Pink, and Green were primarily used prior to July 1965.) These two studies are the Ranch Hand study (Lathrop et al., 1984) and the Center for Disease Control's (CDC) study of birth defects (Erickson et al., 1984). A third study examines the birth defects in children born to Australian Vietnam veterans (Donovan et al., 1983).

The Ranch Hand study (Lathrop et al., 1984) compared airmen who had flown in the aerial spraying of defoliants (in fixed-wing aircraft) to those who belonged to flying organizations responsible for transporting cargo. Other types of spraying (helicopters and backpacks) were not included in the Ranch Hand operations. While the data in this report have not yet been verified (through birth registration or medical records), preliminary analyses have examined various measures of fertility and reproductive success (through pregnancy outcomes, sperm count, and morphology). The researchers are currently validating the interview data and will subsequently (they state) do more detailed analyses. Exposure to 2,3,7,8-TCDD was estimated by calculating the amount of 2,3,7,8-TCDD (in herbicides) used during each airman's tour and dividing this number by the total number of airmen with the same responsibilities during the subject's tour.

The Ranch Hand study individually matched comparisons to each exposed man, put in replacements for refusals, and did not maintain the matches in the analyses. Due to these procedures, determination of the total study population and response rate is difficult; 1,174 Ranch Handers were included in the analysis of reproductive data, as were 1,531 comparisons. Both the man and his current or former spouse were interviewed on reproductive history; research has

shown such interview data on reproductive events to have greater validity when collected from the wife. However, the researchers combined pregnancies from both spouses (but not necessarily reported by both), so it is impossible to examine the women's data separately. No differences were observed in early or late fetal loss, induced abortion, or live births in a crude analysis of the two groups. More detailed analyses examined enlisted men and officers separately, thus reducing the power of the analyses. Some important independent risk factors were not adjusted for in the analysis (e.g., prior fetal loss in analyses of current fetal loss). It appears that multiple pregnancies per family group were all analyzed; however, there was no discussion of the problems associated with the analysis of nonindependent events. A statistically significant excess was found for birth defects, controlling for parental age and for maternal smoking and drinking; however, the reproductive outcome of "birth defects" is probably the one which most needs validation against medical records to assure accurate classification. No differences were observed in sperm count or morphology in the two groups. The more detailed analysis planned for the future could result in useful information since the potential for misclassification of exposure in this study appears to be less than for the other veterans studies.

Another major study of U.S. Vietnam veterans was a case-referent study of birth defects done by the CDC (Erickson et al., 1984). The cases and referents were drawn from births occurring in metropolitan Atlanta from 1968 through 1980. The 7,133 eligible cases consisted of live and still births with serious or major defects identified using the 8th revision of the International Classification of Diseases (ICD-8). The referent group was selected from metropolitan Atlanta live births during the same years; the referents were

frequency-matched on race, year of birth, and hospital of birth. Power calculations suggested that a referent group of 3,000 was sufficient; above that no substantial increase in power would be found for "all defects." The authors also stated that power for odds ratios greater than 3.0 for specific birth defects also would not be substantially affected by using more comparisons. To allow for a 70% response rate, 4,246 referent births were selected. Seventy percent of the women responded, but only 56% of the men did.

The potential for exposure for the CDC study was based on three definitions: First, a man was considered exposed if he had served in any capacity at any time in Vietnam before the conception of the infant. Second, each veteran was queried about his exposure to Agent Orange. Third, an Exposure Opportunity Index (EOI) score was (subjectively) developed by a panel of specialists who evaluated the veterans' duties, location, and time spent in Vietnam. Veteran status was examined to determine whether any military service might be associated with the 96 birth defects examined. Veteran status was not associated and subsequently dropped from further analyses.

These data were analyzed a number of ways, but for all, the data were stratified on the three variables used for the frequency matching: (1) without potentially confounding characteristics; (2) with key characteristics identified a priori (maternal age, education, and alcohol consumption, and the presence of birth defects in close (first-degree) relatives of the child studied; and (3) with a posteriori testing of 108 other characteristics. In addition, certain groupings of the 96 birth defect categories, thought to be related, were also examined. A limited number of elevated findings were reported out of almost 400 analyses. Spina bifida was associated with both levels of the EOI indexes; cleft lip without cleft palate was associated with

veteran status and high EOI; specified anomalies of nails was reported in Vietnam veterans; and "other neoplasms" were associated with high EOI. Due to the large number of analyses and hypotheses tested, one would expect approximately 20 significant associations (elevated or decreased odds ratios) with a significance level of $p = 0.05$ by chance alone. Only 11 statistically significant findings were reported, less than that expected by chance. The authors appropriately concluded that ". . . the data collected contain no evidence to support the position that Vietnam veterans have had a greater risk than other men for fathering babies with all types of serious structural birth defects combined."

The Australian government examined the association between military service in Vietnam and birth defects in their offspring (Donovan et al., 1983). A case-referent study compared births occurring from 1966 through 1979, of 8,517 children with defects recognizable at birth to an equal number of live born children without birth defects. The referents were matched by time of birth and maternal age. Exposure was defined as presence of the father in Vietnam; no specific index of exposure was developed, but the investigators stated that such exposure was probably low for the Australian troops in Vietnam. No difference was observed in exposure patterns between cases and referents.

For veteran studies, the exposures are to the fathers, typically many years before the pregnancy under study was conceived. These studies have used different methods to assign veterans into different exposure groups, based on service in Vietnam (Erickson et al., 1984; Donovan et al., 1983), matching of troop movement with aerial spraying (Erickson et al., 1984), or participation in the Ranch Hand operations (Lathrop et al., 1984). At the time of these

studies, a suitably sensitive assay for 2,3,7,8-TCDD in serum did not exist. The CDC recently reported on a new assay they developed in 1986; this assay yielded serum 2,3,7,8-TCDD levels which correlated well ($r = 0.98$) with 2,3,7,8-TCDD levels in the adipose tissue of the same individuals (CDC, 1987). Paired samples collected from the same individuals in 1982 and 1987 were used to derive the half-life of the 2,3,7,8-TCDD body burden (6 to 10 years). In this preliminary report, CDC compared the assayed levels of 2,3,7,8-TCDD to the exposure categories they had established using the EOI and interview data: no association was found between the three methods of scoring for potential exposure and the serum levels observed. Furthermore, in their examination of the sera of Vietnam era veterans (444 Vietnam veterans versus 75 non-Vietnam veterans), the median sera levels did not differ for these two subgroups. These data raise a number of issues in the consideration of this body of research:

(1) The relatively long half-life broadens the range of potential mechanisms that could occur if an association exists between paternal 2,3,7,8-TCDD exposure and birth defects. Friedman (1984) and Hatch and Stein (1986) have suggested that such an exposure would cause birth defects in offspring through gene or chromosomal mutation of spermatogonial stem cells (premeiotic effects). However, the long half-life observed means that postmeiotic effects on sperm are also possible. The long half-life also lends more support to Friedman's suggestion of maternal/fetal exposures from 2,3,7,8-TCDD in seminal fluid.

(2) These data suggest that a great deal of misclassification of exposure occurred in the assignment of veteran's to their exposure categories for the CDC study. Plus use of this assay would improve exposure estimation

in the other studies.

(3) Finally, the levels and similarity of median 2,3,7,8-TCDD sera values in both Vietnam and non-Vietnam veterans raises questions about the magnitude of exposure of Vietnam veterans. Other data suggest that the exposures of Vietnam veterans were less than originally thought: the median levels of 2,3,7,8-TCDD in both groups are below those found in the adipose tissue of the control group of a study in eastern Missouri (Patterson et al., 1986).

OCCUPATIONAL STUDIES

Occupational studies tend to be more useful in the evaluation of a potentially toxic exposure in risk assessment. This is especially true for qualitative risk assessment, since one can be fairly certain that exposure to the worker did occur. If good historical industrial hygiene data are available, these can also be of use in quantitative risk assessment. As described in the Vietnam veterans studies, the exposures evaluated here are primarily to male workers and the studies have been restricted to examination of paternal effects; however, wives may have been exposed indirectly, through handling their husband's clothes, etc. Unlike the veterans studies, the exposures may be occurring concurrently with conception, thus increasing the potential exposure level at the time of the pregnancy.

Smith et al. (1982) identified 616 male chemical applicators from a list maintained by New Zealand's agricultural Chemicals Board and 531 comparison workers at small agricultural contracting companies. A total of 89% of the chemical applicators and 83% of the contractual workers responded to a mailed

questionnaire requesting occupational histories from the men and reproductive histories from their wives. Exposure to 2,4,5-T was defined as having sprayed this pesticide during the year of the pregnancy studied or during the year preceding that pregnancy. Group 1 (not exposed to any spraying) consisted of 392 pregnancies; group 2 (sprayed other chemicals than 2,4,5-T) had 109 pregnancies; and group 3 (sprayed 2,4,5-T) had 473 pregnancies. Comparisons of group 3 to group 1 found no statistically significant differences in fetal loss or birth defects. Other risk factors did not appear to be controlled in the analysis of these data: for example, the analysis of miscarriages did not appear to control for maternal age or occurrence of prior fetal loss. Multiple pregnancies per family unit were studied; however, the authors did not address the problem of nonindependent events. Additionally, the power of this study was very low for birth defects.

Dow Chemical studied 930 male workers potentially exposed to dioxins, through work with chlorophenol processes, for at least one month from January 1939 through December 1975 (Townsend et al., 1982). The reproductive experience of their wives, obtained by interview, was compared to the wives of an equal number of unexposed male employees. For the 930 exposed workers, 586 wives were identified and 370 responded (63%); for the comparison group, 559 wives were identified and 345 responded (62%). Exposure potentials from low to high were assigned to jobs using historic surface contamination data by an industrial hygienist familiar with the history of the facility. A pregnancy was considered exposed if the employee had worked in an exposed area for at least one month at any time prior to conception. Thus, this study, in its analysis, has similar problems to those described above for the study of Vietnam veterans concerning the time delay between exposure and conception.

The outcomes examined included miscarriages (< 20 weeks gestation), stillbirths, and birth defects identified (or potentially identified) before the child's first birthday. The relationships of these outcomes were compared to several classifications of exposure: any dioxin, only 2,3,7,8-TCDD, moderate and higher levels of 2,3,7,8-TCDD only, other dioxins only. The "any dioxin" and "only 2,3,7,8-TCDD" categories were done as broad groups, plus split into two groups each, based on duration of exposure ($\leq$ 12 months, or > 12 months) during the entire employment period preceding conception. Over two thousand pregnancies were in the "non-dioxin" group, while the "dioxin" group consisted of 737 pregnancies. The "non-dioxin" group included pregnancies occurring before exposure for exposed workers. No differences were found in the two groups. The power was low for the examination of birth defects. As noted above, the assumption of any exposure greater than one month at any time prior to conception could obscure a true effect in this population. A reanalysis of these data, looking at possible associations with paternal exposure during the 3 to 4 months preceding conception might give more insight into potential paternal effects associated with this exposure.

In a clinical, cross-sectional study, reproductive characteristics in current (N = 131) and retired (N = 161) men who had worked with 2,4,5-T were selected for comparison to workers without exposure (N = 133). Of the workers selected, 55% responded. Historic exposure data were not available, and job titles were not sufficient to estimate exposure; therefore, interview exposure history was used to group workers into a probable exposure category. Contamination of the plant with 2,4,5-T occurred in 1949. For the purpose of comparing reproductive histories, reported chloracne was used to distinguish groups. No differences were found for fetal loss or birth defects between the

workers with chloracne (N = 107/number of pregnancies = 235) and those without (N = 91/number of pregnancies = 203) during or after 1948 (when 2,4,5-T was first handled at the plant under study). Differences were observed in reports of decreased libido and difficulty with erection or ejaculation. Due to the very indirect nature of exposure assessment, the probability of misclassification of the exposure category is great.

AGRICULTURAL AND FORESTRY SPRAYING

A number of studies have examined the relationship between reproductive effects and aerial spraying of pesticides (Field and Kerr, 1979; Hanify et al., 1981; Nelson et al., 1979; Thomas, 1980). In these studies, exposures could be to either or both parents. These are all ecologic studies, in which exposure of study members is assumed by their presence in an area (e.g., by their residence), with a specified likelihood of exposure to a given agent. These studies are heir to the "ecologic fallacy" in that individuals defined by residence or some other factor as being in a certain exposure category, may not in fact belong in that category. Thus, such studies are of very limited value in either qualitative or quantitative risk assessments; therefore, only brief discussions of published reports of these follow.

The earliest of these studies (Nelson et al., 1979) examined the association between cleft lip and/or cleft palate (CL/P) in live births occurring during the 32-year period beginning in 1943 and the spraying of 2,4,5-T in Arkansas. Approximately 1,200 cases of CL/P were identified using both birth certificate data and records of the Crippled Children's Services of Arkansas Social and Rehabilitative Services. 2,4,5-T was primarily used on

rice; therefore exposure was determined by estimating the proportion of rice acreage to total acreage in each county from data supplied by the Arkansas State Plant Board (1970-1974). (The exposure definition does not address the use of 2,4,5-T in forestry.) The 75 counties were then divided into categories of low, medium, and high potential for 2,4,5-T exposure. An increase was observed in facial clefts over time, which the authors suggested were related to improved recording and case ascertainment and not to an association with 2,4,5-T spraying.

A letter to the editor of Lancet at approximately the same time (Field and Kerr, 1979) discussed the relationship between 2,4,5-T and 2,3,7,8-TCDD and neural-tube defects in New South Wales, Australia. Only limited data were presented in this letter: in New South Wales, annual rates for neural-tube defects (anencephaly and meningomyelocele) were compared to annual usage figures for 2,4,5-T for all of Australia. Seasonal variation of 2,4,5-T usage was obtained by questionnaire of local governments from the years 1965-1976. 2,4,5-T usage increased from 90 tonnes (1 tonne = 1,000 kg) in 1965 to 482 tonnes in 1976. The authors reported a linear correlation between the previous years' 2,4,5-T usage and neural-tube defects. In addition, a seasonal pattern was noted, with the highest rates in conceptions occurring during the summer months (December, January, February). The authors also noted that in the Northern Hemisphere, the highest rates in conceptions also occurred during the summer months. In addition to the limitations present in ecologic studies, no comparison rates of neural-tube defects were reported in communities without such exposure, nor were high versus low exposure communities compared.

Another letter to the editor of Lancet the next year (Thomas, 1980) discussed a comparison of selected birth defects (cleft lip, cleft palate,

spina bifida, anencephalus, and cystic kidney disease) and use of 2,4,5-T in Hungary. The birth defects were identified using Hungary's malformation registry, which records malformations recognized at up to one year of age. In Hungary, the use of 2,4,5-T rose from 46 tonnes in 1969 to over 1,200 tonnes in 1975. The rates of some birth defects (spina bifida and anencephalus) decreased over the time period examined, while others (cleft lip, cleft palate, and cystic kidney disease) remained essentially unchanged. In a comparison of this report with the New South Wales letter from Field and Kerr (1979), Thomas noted that: (1) the population examined in Australia was spread over a much greater geographic area (thus reducing the probability for exposure), (2) over half of the population in Hungary lived in rural areas, while only 15% of the population in Australia did, and (3) 24.6% of the population in Hungary was actively involved in forestry and agriculture as compared with only 7.4% of the Australian population. While these data suggest no association between the defects and 2,4,5-T, this report also did not attempt to compare exposed communities with less exposed (or unexposed) groups.

Hanify et al. (1981) compared rates of diagnosed birth defects in stillbirths ($\geq$ 28 weeks gestation) and live births in Northland, New Zealand, to densities of aerial 2,4,5-T spray application (1960-1977). The reproductive events were identified through seven regional hospital records: 37,751 births which included 436 stillbirths, 264 neonatal deaths, and 510 with recorded birth defects. The location, data, and quantity of 2,4,5-T sprayed was obtained from company records, and monthly estimates were made for each of the seven regions. Environmental levels were estimated modeling both the new applications plus that fraction of the previous applications thought to still be present. 2,4,5-T was not sprayed from 1959-1965; thus, for this time

period, exposure was considered to be zero. The zero time period (1960-1965) was compared to years thought to be representative of "spraying years" (1972-1976). Associations were found for all birth defects and for talipes under certain assumptions of environmental persistence of 2,4,5-T. The authors did not discuss the possibility of secular trends in the data, due to other medical or environmental factors, or differences in the identification/recording of malformations over time.

OTHER ENVIRONMENTAL EXPOSURES -- SEVESO, ITALY

This category of "other environmental exposures" includes unplanned exposures such as exposures from accidental emissions from industrial facilities. With an immediately recognizable environmental incident, exposure of interest for reproduction could occur for both parents, or just for the mother, if she is already pregnant at the time of first exposure (unless there could be in utero exposure to dioxin in seminal fluid). In 1976, during the production of trichlorophenol at the ICMESA plant in Seveso, Italy, a runaway reaction resulted in an explosion that ultimately contaminated 700 acres in the surrounding community. Environmental levels of 2,3,7,8-TCDD were determined using wipe tests, evaluating toxic effects in small animals, and analyzing grass samples. Approximately 2 weeks later, over 200 families were evacuated from high contamination areas. Exposures were sufficiently high for chloracne to be observed in this environmentally exposed population. Several reports (Bisanti et al., 1979; Homberger et al., 1979; Pocchiari, 1980; Pocchiari et al., 1980; Reggiani, 1978; Rehder et al., 1978; Tuchmann- Duplessis, 1977) reported on comparisons of four potentially affected communities (Seveso, Meda,

Cesano, and Desio) to nearby, unexposed communities. Changes in fetal loss rates occurring during the last quarter of 1976 and first quarter of 1977 were found in both exposed and unexposed communities. An estimated 150 women were in the first trimester of pregnancy at the time of the accident (Rehder et al., 1978); of these, 125 women wished therapeutic abortions by October 1976. Therapeutic abortions were approved for 30. Another estimate (Tuchmann-Duplessis, 1980) reports a total of 108 (50 in 1976 and 58 in 1977) therapeutic abortions in the four affected communities. Several reports (Bisanti et al., 1979; Pocchiari, 1980; Pocchiari et al., 1980) suggested that a large number of women obtained unapproved, and therefore not reported, therapeutic abortions. This supposition was supported by a steep decrease in birth rates in the first 6 months of 1977, primarily observed in the exposed communities. (All communities had had decreases over time; however, the decrease in exposed communities at this key time was much larger.) Marked increases in the number of birth defects were noted in 1977. These have been attributed to changes in reporting. Prior to this time, only certain birth defects were required to be reported to Italian Health Officers (Reggiani, 1980). In addition to the limitations due to legal requirements, there were other reasons for the underreporting of malformations: "Traditionally, Italian physicians have under-reported congenital malformations because of their severe negative social implications" (Tuchmann-Duplessis, 1980a, b). Although physicians and midwives were encouraged to do more complete recording, Reggiani (1978) concluded that the malformation data were "missing" for 1976 and "incomplete" for the first trimester of 1977. In general, the researchers cited above felt that, in spite of the problems associated with the data, adverse reproductive effects did not occur in this population. However, the

data do not appear to be complete enough to make definitive conclusions. In addition, these reports have the same problems concerning exposure assessment as those described previously in the Agricultural/Forestry Spraying section.

STUDIES OF THE VIETNAMESE

While studies of the Vietnamese have not been published in Western journals, a review has been presented by Constable and Hatch (1985). Two types of studies have been executed: In the South, the reproductive experience of exposed and unexposed couples was compared; in this case, effects of maternal and paternal exposures cannot be separated. In the North, reproductive experience was compared for families of men who had served in the South (thus, assuming paternal exposure) to families of men remaining in the North (and therefore assuming no exposure of the father). Exposures for these studies have been defined by residence, using historical data on spraying and/or determined by the evidence of destruction of vegetation. Thus, these studies have the same limitations for use in qualitative and quantitative risk assessment as discussed previously.

Only one study (Lang et al., 1983 described in Constable and Hatch, 1985) has attempted to determine types of exposure (e.g., exposed during spraying versus the more indirect exposure due to dust or diet) and assign exposure scores. In this study of veterans in the North, exposure was restricted to the father at some time prior to conception, thus resulting in the same problems encountered in the American and Australian studies of veterans.

SUMMARY

All three Vietnam veterans studies examined the occurrence of birth defects. The Ranch Hand study found an excess for "all birth defects" (these were not validated); the CDC study found a difference in exposure for cases with spina bifida, cleft lip without palate, specified anomalies of the nails, and "other neoplasms"; the third study (Donovan et al., 1983) found no differences. No associations with other reproductive outcomes were found in the Ranch Hand study (the only study of the three that looked at the other outcomes). No differences were found in reproductive outcome in the three workplace studies (Moses et al., 1984; Smith et al., 1982; Townsend et al., 1982), however, Moses et al. did report increased impotence and sexual dysfunction in 2,4,5-T workers with chloracne (assumed to result from 2,4,5-T and/or 2,3,7,8-TCDD). Several of the ecologic studies (Nelson et al., 1979; Field and Kerr, 1979; Hanify et al., 1981) reported associations with some birth defects and volume of 2,4,5-T application; these data were contested by Thomas (1980). Birth defects in Seveso increased after the plant accident, potentially due to changes in reporting (Reggiani, 1980). As presented in this paragraph, one might conclude that 2,3,7,8-TCDD/2,4,5-T is related to adverse reproductive outcomes, but as discussed previously, limitations in the design of the studies neither allow nor rule out such an interpretation.

Due to the very nature of the exposures to 2,3,7,8-TCDD, obtaining suitable data for either a qualitative or quantitative risk assessment is difficult, if not impossible. First, the exposures are primarily in general environmental settings (wartime exposures are, for these purposes, being classified as environmental) where it is difficult to define whether an

individual is even exposed and nearly impossible to quantitate that exposure. In occupational settings, both qualitative and quantitative exposure data may exist, but small population sizes limit the power of the studies. A nationwide study planned by the National Institute for Occupational Safety and Health (NIOSH) on their Dioxin Registry could overcome the problems of limited power.

All of the studies described above contained a major limitation: imprecise definitions of exposure of the subjects. In many studies, the exposure was assumed by place of residence or presence in a particular area. Even where more descriptive exposure data are available, such as the CDC and Ranch Hand studies, misclassification may be great (CDC, 1987). Other studies, such as the ecologic studies, compared the same area over time rather than to a comparison area (e.g., Field and Kerr, 1979; Thomas, 1980); thus, differences in these studies may be due to differences in secular trends rather than to changes in potential exposure to 2,3,7,8-TCDD. The evidence from all of these studies, therefore, is open to question.

The reports with more detailed exposure information include those examining paternal exposure. Kimmel has reviewed the animal literature in Appendix C and reported that 2,3,7,8-TCDD resulted in testicular atrophy and reduced sperm count, but a dominant lethal study found no effects (Kimmel, 1988). The animal data are too limited, at this time, to suggest the presence or absence of an effect in the offspring in studies with human male exposure. The large differences in time frame between male exposure and conception in the epidemiologic studies probably reduces the chance of identifying any paternally mediated effect that is potentially occurring. However, new data on the half-life of 2,3,7,8-TCDD in humans (CDC, 1987) suggests some exposure may be occurring many years later, depending on the level of initial exposure.

Reanalyses of occupational studies, with stricter exposure definitions, might yield useful information.

The only studies that address the possibility of female exposure are the ecologic/environmental studies and the studies of Seveso. In his review of the animal literature, Kimmel (1988) found evidence of reproductive and developmental effects of dioxin. The human studies have less informative exposure data, and so are less useful in drawing conclusions concerning the reproductive effects of 2,3,7,8-TCDD. Additionally, in both types of studies, males may also be exposed, thus clouding the interpretation of such data.

REFERENCES

Bisanti, L.; Bonetti, F.; Caramaschi, F.; Del Corno, G.; Favaretti, C.; Giambelluca, S.; Marni, E.; Montesarchio, E.; Puccinelli, V.; Remotti, G.; Volpato, C.; Zambrelli, E. (1979) Experience of the accident of Seveso. Proceedings of the 6th European Teratology Conference, Akademiai Kiado. Pub.

Centers for Disease Control (CDC). (1987) Serum dioxin in Vietnam-era veterans - preliminary report. Morbidity and Mortality Weekly Report. 36(28):470-475.

Constable, J.D.; Hatch, M.C. (1985) Reproductive effects of herbicide exposure in Vietnam: recent studies by the Vietnamese and others. Teratogenesis Carcinog. Mutagen. 5(4):231-250.

Donovan, J.W.; Adena, M.A.; Rose, G.; Batistutta, D. (1983) Case-control study of congenital anomalies and Vietnam service. Canberra: Australian Government Publishing Services. (As described in Hatch and Stein, 1986).

Erickson, J.D.; Mulinare, J.; McClain, P.W.; Fitch, T.G.; James, L.M.; McClearn, A.B.; Adams, M.J. (1984) Vietnam veterans' risks for fathering babies with birth defects. JAMA 252(7):903-912.

Field, B.; Kerr, C. (1979) Herbicide use and incidence of neural-tube defects. Lancet 1(8130):1341-1342.

Friedman, J.M. (1984) Does agent orange cause birth defects? Teratology 29:193-221.

Hanify, J.A.; Metcalf, P.; Nobbs, C.L.; Worsley, R.J. (1981) Aerial spraying of 2,4,5-T and human birth malformations: an epidemiological investigation. Science 212:349-351.

Hatch, M.C. (1984) Reproductive effects of the dioxins. In: Lowrance, W.W., ed. Public health risks of the dioxins. Proceedings of a symposium held at The Rockefeller University in New York City, Oct. 19-20, 1983. Los Altos, CA: William Kaufmann.

Hatch, M.C.; Stein, Z.A. (1986) Agent orange and risks to reproduction: the limits of epidemiology. Teratogenesis Carcinog. Mutagen. 6(3):185-202.

Homberger, E.; Reggiani, G.; Sambeth, J.; Wipf, H. (1979) The Seveso accident: its nature, extent and consequences. Report from Givaudan Research Company Ltd. and F. Hoffmann-La Roche & Co. Ltd.

Kimbrough, R.D.; Falk, H.; Stehr, P.; Fries, G. (1984) Health implications of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) contamination of residential soil. J. Toxicol. Environ. Health 14(1):47-93.

Kimmel, G.L. (1988) Reproductive and developmental toxicity of 2,3,7,8-TCDD. In: A cancer risk-specific dose estimate for 2,3,7,8-TCDD, Appendix C. External Review Draft. U.S. Environmental Protection Agency, Washington, DC.

Lathrop, G.D.; Wolfe, W.H.; Albanese, R.A.; Moynahan, P.M. (1984) An epidemiologic investigation of health effects in air force personnel following exposure to herbicides: baseline morbidity study results. USAF School of Aerospace Medicine, Brooks Air Force Base, Texas.

Moses, M.; Lillis, R.; Crow, K.D.; Thornton, J.; Fischbein, A.; Anderson, H.A.; Selikoff, I.J. (1984) Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. Am. J. Ind. Med. 5:161-182.

Nelson, C.J.; Holson, J.F.; Green, H.G.; Gaylor, D.W. (1979) Retrospective study of the relationship between agricultural use of 2,4,5-T and cleft palate occurrence in Arkansas. Teratology 19:377-384.

Patterson, D.G.; Hoffman, R.E.; Needham, L.L.; Roberts, D.W.; Bagby, J.R.; Pirkle, J.L.; Falk, H.; Sampson, E.J.; Houk, V.N. (1986) 2,3,7,8-Tetrachlorodibenzo-p-dioxin levels in adipose tissues of exposed and control persons in Missouri: an interim report. JAMA 256(19):2683-2686.

Pocchiari, F. (1980) Accidental TCDD contamination in Seveso (Italy): epidemiological aspects. FIFRA Docket No. 415, Exhibit 1469. U.S. Environmental Protection Agency, Washington, DC.

Pocchiari, F.; Silano, V.; Zampieri, A. (1980) Human health effects from accidental release of TCDD at Seveso (Italy). FIFRA Docket No. 415, Exhibit 1470. U.S. Environmental Protection Agency, Washington, DC.

Rappe, C. (1985) Problems in analysis of PCDDs and PCDFs and presence of these compounds in human milk. Consultation on organohalogen compounds in human milk and related hazards. Geneva: World Health Organization.

Reggiani, G. (1978) Medical problems raised by the TCDD contamination in Seveso, Italy. Arch. Toxicol. 40:161-188.

Reggiani, G. (1980) Direct testimony before the U.S. Environmental Protection Agency. FIFRA Docket No. 415, Exhibit 861. U.S. EPA, Washington, DC.

Rehder, H.; Sanchioni, L.; Cefis, F.; Gropp, A. (1978) Pathological-embryological investigations in cases of abortion related to the Seveso accident. Journal of Swiss Medicine 108(42):1617-1625.

Schecter, A.; Ryan, J.J.; Constable, J.D. (1987) Polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran levels in human breast milk from Vietnam compared with cow's milk and human breast milk from the North American continent. Chemosphere 16(8/9):2003-2016.

Smith, A.H.; Fisher, D.O.; Pearce, N.; Chapman, C.J. (1982) Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Arch. Environ. Health 37(4):197-200.

Thomas, H.F. (1980) 2,4,5-T use and congenital malformation rates in Hungary. Lancet ii:214-5.

Townsend, J.C.; Bodner, K.M.; Van Peenen, P.F.; Olsen, R.D.; Cook, R.R. (1982) Survey of reproductive events of wives of employees exposed to chlorinated dioxins. Am. J. Epidemiol. 115(5):695-713.

Tuchmann-Duplessis, H. (1977) Embryo problems posed by the Seveso accident. Le Concours Medical No. 44.

Tuchmann-Duplessis, H. (1980a) Direct testimony before the U.S. Environmental Protection Agency. FIFRA Docket No. 415, Exhibit 864. U.S. EPA, Washington, DC.

Tuchmann-Duplessis, H. (1980b) Tables in direct testimony before the U.S. Environmental Protection Agency. FIFRA Docket No. 415, Exhibit 864a. U.S. EPA, Washington, DC.

U.S. Environmental Protection Agency. (1985) Health assessment document for polychlorinated dibenzo-p-dioxins. Office of Health and Environmental Assessment, Washington, DC. EPA/600/8-84/014F. NTIS PB86-122546/AS.

U.S. Environmental Protection Agency. (1988) Estimating exposures to 2,3,7,8-TCDD. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington, DC. External Review Draft. EPA/600/6-88/005A.

van den Berg, M.; van der Wielen, F.W.M.; Olle, K.; Van Boxtel, C.J. (1986) The presence of PCDDs and PCDFs in human breast milk from the Netherlands. Chemosphere 15(6):693-706.

March 1988
Review Draft

APPENDIX E

IMMUNOTOXICITY OF 2,3,7,8-TCDD: REVIEW, ISSUES, AND UNCERTAINTIES

Babasaheb R. Sonawane, Ph.D.
Reproductive Effects Assessment Group
Office of Health and Environmental Assessment

Ralph J. Smialowicz, Ph.D.
Health Effects Research Laboratory
Office of Health Research

Robert W. Luebke, Ph.D.
Health Effects Research Laboratory
Office of Health Research

Office of Research and Development
U.S. Environmental Protection Agency

EXECUTIVE SUMMARY

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is a potent immunosuppressant in the laboratory animal species studied; however, the immunological effects are apparent at exposure levels that also produce other discernible pathological lesions and reproductive/developmental effects. There are no unequivocal cases of significant immune function alterations in humans following exposure to 2,3,7,8-TCDD. The cellular and molecular mechanism(s) of 2,3,7,8-TCDD-induced immunotoxicity is unknown. Significant data gaps and uncertainties exist to prevent an immunotoxicity-based health hazard evaluation.

INTRODUCTION

This document discusses the relevant scientific literature on the immunotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) in laboratory animals and humans. The document does not provide a comprehensive literature review of the 2,3,7,8-TCDD-induced immune effects; however, attempts were made to identify and discuss critical studies and issues, strengths and weaknesses of the data, and significant uncertainties associated with the evaluation and interpretation of the data. Furthermore, significant data gaps in knowledge are recognized as they may relate to potential risk to the immune system of humans upon exposure to 2,3,7,8-TCDD.

The immunotoxic effects of 2,3,7,8-TCDD have been studied by numerous investigators for over a decade. Several recent reviews have been published that provide a very good overview of the animal and human data dealing with the immune alterations following exposure to 2,3,7,8-TCDD (Dean and Lauer, 1984; Dean and Kimbrough, 1986; Thomas and Faith, 1985). The U.S. Environmental

Protection Agency also reviewed the immunological effects of 2,3,7,8-TCDD in the Health Assessment Document for Polychlorinated Dibenzo-p-Dioxins (U.S. EPA, 1985). The present review updates the immunotoxicity literature on 2,3,7,8-TCDD and presents issues of concern and uncertainties in risk assessment.

ANIMAL STUDIES

Early experimental work with 2,3,7,8-TCDD revealed that the thymus is a target organ of toxicity. All animal species that have been studied have consistently displayed involution of the thymus and loss of cortical thymocytes following acute or chronic exposure to 2,3,7,8-TCDD, as well as lymphocyte depletion of T-cell areas of the lymph nodes and the spleen (Harris et al., 1973; Zinkl et al., 1973; Gupta et al., 1973; Vos et al., 1973; 1974; Luster et al., 1979; 1982). While these changes in the lymphoid tissues were similar to those produced by glucocorticosteroids, adrenalectomy failed to prevent 2,3,7,8-TCDD-induced thymic atrophy or hepatotoxicity (van Logten et al., 1980). The administration of thymosin to mice exposed to 2,3,7,8-TCDD by postnatal maternal treatment did not affect thymus atrophy or decrease mitogen responses (Vos et al., 1978). Thyroidectomy, however, was found to protect rats from the immunosuppressive effects of 2,3,7,8-TCDD (Pazdernik and Rozman, 1984). Vos and co-workers (1973) reported the effects of 2,3,7,8-TCDD on the immune system of guinea pigs, rats, and mice. Several tests were employed in these three species, and the authors concluded that the effect of 2,3,7,8-TCDD was primarily on the cell-mediated immune function of these animals. Guinea pigs treated with 1 ug/kg of 2,3,7,8-TCDD per week either died or were moribund after four doses. At this dose and also at lower doses (0.008, 0.04, and 0.2 ug/kg), guinea pigs showed severe loss of body weight, depletion of the lymphoid organs, particularly

the thymus, and lymphopenia. At sublethal doses, the effects were primarily on the thymus. The weights of other lymphoid organs, such as the spleen, cervical lymph nodes, and popliteal lymph nodes, were not affected. Adrenals showed a slight enlargement at the 0.2 ug/kg dose level. Serum cortisol and corticosterone values were not influenced by the 2,3,7,8-TCDD treatment. No microscopic lesions were apparent in any of the lymphoid organs upon dosing with up to 0.2 ug/kg of 2,3,7,8-TCDD per week other than atrophy of the thymic cortex (Vos et al., 1973).

In selected experiments, the treated and control animals were sensitized to tetanus toxoid or killed Mycobacterium tuberculosis. Seven days after the tetanus toxoid injection, there was a small but significant increase in the serum antitoxin levels at 0.008 and 0.04 ug/kg dose levels. The animals were challenged by a second dose of tetanus toxoid 2 weeks after the first challenge to evaluate the secondary response. One and 2 weeks after the second dose, the guinea pigs treated with 0.2 ug/kg of 2,3,7,8-TCDD per week had significantly lower tetanus antitoxin titers. The animals challenged with M. tuberculosis were tested for their skin reactivity 12 and 19 days later by intradermal tuberculin injections. Both skin thickness and the diameter of the reaction site were significantly decreased, the former being significant at the 0.04 ug/kg dose level (Zinkl et al., 1973; Vos et al., 1973).

Female albino rats were treated orally with 0.2, 1, and 5 ug/kg of 2,3,7,8-TCDD once a week for 6 weeks. The body weights and thymus weights were reduced at the highest dose level and adrenal weights were reduced at the 1 and 5 ug/kg dose level. The relative thymus weight (organ/body weight ratio) was approximately half that of the control animals at the 5 ug/kg dose level, whereas at the same dose level, the relative spleen weights were significantly increased. The total peripheral blood leukocyte count and lymphocyte numbers

did not show a significant 2,3,7,8-TCDD-related effect as observed in guinea pigs. Skin reactivity of rats challenged to tuberculin was not affected at any 2,3,7,8-TCDD dose levels (Zinkl et al., 1973; Vos et al., 1973).

Graft versus host (GVH) activity of mice was evaluated after 0.2, 1, 5, and 25 ug/kg of 2,3,7,8-TCDD treatment per week for 4 weeks. The absolute and relative thymus weights were decreased in groups treated with 5 ug/kg of 2,3,7,8-TCDD. Dose-related reduction in GVH activity was observed (Vos et al., 1973).

Mice treated orally with 2,3,7,8-TCDD were challenged with either Salmonella bern or Herpesvirus suis (Thigpen et al., 1975). In two separate experiments the animals were exposed to 2,3,7,8-TCDD levels ranging from 0.5 to 20 ug/kg once every week for 4 weeks. The animals were challenged with the infectious agents one day after the fourth dose of 2,3,7,8-TCDD. Treatment of mice with 2,3,7,8-TCDD reduced the time of death after the bacterial challenge at the 5 ug/kg dose level whereas the increased mortality was obvious at 1 ug/kg. The challenge with H. suis influenced neither the period of time to death nor the mortality rate.

Vos and co-workers (1978) reported the response of young mice to Escherichia coli endotoxin after 2,3,7,8-TCDD treatment. There was a dose-related increase in mortality, both with respect to 2,3,7,8-TCDD and the endotoxin. While the administration of 250 ug of endotoxin produced no mortality in the control group, as little as 10 ug of endotoxin produced death in mice treated with 15 or 50 ug 2,3,7,8-TCDD. A similar increase in endotoxin sensitivity was reported when mice were treated with a single oral dose of 100 ug/kg of 2,3,7,8-TCDD and challenged with 20 ug of endotoxin. In these experiments, a 2,3,7,8-TCDD dose-related decrease in body, thymus, and spleen weights was observed.

The increased susceptibility to S. bern reported in mice exposed to 2,3,7,8-TCDD (Thigpen et al., 1975) has been postulated to be due to an increased sensitivity of 2,3,7,8-TCDD-exposed mice to bacterial endotoxin (Vos et al., 1978), although variable effects have been reported in host resistance following 2,3,7,8-TCDD exposure (Dean and Lauer, 1984; Vos et al., 1978; Luster et al., 1980). Other studies have shown a correlation between increased mortality from bacterial or parasitic infections and decreased serum complement levels (White et al., 1986) or decreased B-cell mediated responses (Tucker et al., 1986) in 2,3,7,8-TCDD-exposed mice, respectively.

Clark et al., (1981) have shown that low doses of 2,3,7,8-TCDD (4 ng/kg to 0.4 ug/kg) administered intraperitoneally once a week for 4 weeks suppress the generation of cytotoxic T-cells by lymph node and spleen cells in male C57BL/6 mice but have little effect on delayed hypersensitivity, antibody responses, or thymic cellularity. Suppressor cells capable of blocking the cytotoxic T-cell response were found in the thymus of 2,3,7,8-TCDD-treated mice following cumulative doses of 2,3,7,8-TCDD as low as 4 ng/kg. The immunosuppressive effects observed at 4 ng/kg were significantly less than the 4 ug/kg required to induce thymic atrophy. In 1983, the same investigators (Clark et al., 1983) reported that susceptibility to 2,3,7,8-TCDD-induced immunosuppression in mice is strain-dependent, and occurs at doses that have little effect on hepatic microsomal enzymes. Clark et al. also reported that other types of haloaromatics can induce similar immunosuppression provided they possess sufficient binding affinity for the genetically controlled 2,3,7,8-TCDD-receptor protein. These observations suggest a receptor-dependent mechanism for the stimulation of suppressor T-cell activity by haloaromatic hydrocarbons.

Examination of T-cell-mediated responses in 2,3,7,8-TCDD-exposed animals has generally demonstrated a correlation between thymic atrophy and impaired

cell-mediated immunity. Exposure of mice, rats, and/or guinea pigs to 2,3,7,8-TCDD has been reported to result in depressed delayed hypersensitivity (Vos et al., 1973; Faith and Moore, 1977; Clark et al., 1981), prolonged allograft rejection (Vos and Moore, 1974), depressed GVH response (Vos and Moore 1974), decreased responses to T-cell mitogens and/or allogeneic cells in vitro (Dean and Lauer, 1984; Vos and Moore, 1974; Luster et al., 1980), and decreased generation of cytotoxic T lymphocytes (CTL) (Clark et al., 1981, 1983). Depressed CTL activity in adult mice following exposure to 2,3,7,8-TCDD has been reported to be associated with an increase in suppressor T-cells, but not associated with a reduction in the frequency of CTL precursors (Clark et al., 1981; 1983; Nagarkatti et al., 1984).

Nonspecific immune responses affected by natural killer cells (Dean and Lauer, 1984; Mantovani et al., 1980) and macrophages (Dean and Lauer, 1984; Vos et al., 1978; Mantovani et al., 1980) have not been observed to be affected by 2,3,7,8-TCDD exposure. Both of these cell types possess tumoricidal, bactericidal, and virucidal activity.

2,3,7,8-TCDD has also been reported to affect bone marrow and humoral immune responses of experimental animals. Exposure of mice to 2,3,7,8-TCDD resulted in myelotoxicity and suppression of bone marrow progenitor cells (Tucker et al., 1986; Luster et al., 1985; Chastain and Pazdernik, 1985). Studies in mice exposed to 0, 1.0, 5.0, or 15 ug/kg body weight of 2,3,7,8-TCDD pre- and postnatally by maternal dosing indicated that both 5 and 15 ug/kg dosage groups had a significant reduction in bone marrow cellularity, (colony-forming unit-spleen (CFU-S) or pluripotent stem cells and colony-forming unit-granulocyte/macrophage (CFU-GM). Hematology profiles and blood smears revealed a normocytic anemia in these mice (Luster et al., 1980). Bone marrow toxicity correlated with depressed immunologic and host-resistance responses.

The hematopoietic stem cells have a limited renewal capacity, and damage to these cells can induce a permanent decrease in their proliferative capacity; however, this limited evidence of bone marrow toxicity, as observed in mice, is difficult to evaluate due to the lack of additional significant studies in other species or humans.

The antibody response of 2,3,7,8-TCDD-exposed mice, as measured by the plaque-forming cell (PFC) assay, was suppressed following immunization with T-cell dependent and/or T-cell independent antigens (Tucker et al., 1986; Vecchi et al., 1983; Holsapple et al., 1986). Furthermore, these effects on B-cell-mediated responses were observed to occur at 2,3,7,8-TCDD doses below those that cause thymic atrophy (van Logten et al., 1980; Tucker et al., 1986; Luster et al., 1985; Vecchi et al., 1983; Holsapple et al., 1986).

Susceptibility to suppression of humoral immune responses and 2,3,7,8-TCDD induction of the aryl hydrocarbon hydroxylase (AHH) system were found to correlate (Luster et al., 1980; Tucker et al., 1986; Chastain and Pazdernik, 1985; Vecchi et al., 1983; Holsapple et al., 1986), as does thymic atrophy (Poland and Glover, 1980), T-cell-mediated responses (Nagarkatti et al., 1984), and serum complement levels (White et al., 1986). A good correlation between the degree of AHH inducibility and immunosuppression was observed in F_1 crosses of "responsive" (Ah^b/Ah^b) and "nonresponsive" (Ah^dAh^d) mice (Nagarkatti et al., 1984; Vecchi et al., 1983). These results, taken together, indicate that there is a strong association between the presence of the Ah receptor and the induction of immune effects following 2,3,7,8-TCDD exposure in experimental animals, and susceptibility to 2,3,7,8-TCDD-induced immune effects in the mouse may be under genetic influences.

The immunological reactivity of young or/suckling rats and mice has been evaluated using a variety of experimental protocols employing prenatal or post-

natal exposure of mothers or a continued prenatal through postnatal exposure with 2,3,7,8-TCDD. One such study reported by Vos and Moore (1974) is summarized below. Pregnant rats were treated orally with 1 or 5 ug/kg body weight of 2,3,7,8-TCDD on day 11 and 18 of gestation. The weights of 1-day-old pups from mothers treated with 5 ug/kg dose level were significantly reduced. At this dose level, reduced weights of the thymus and spleen were also observed. Most of the pups in the high-treatment group died within 25 days after birth. The newborn animals from mothers given a 1 ug/kg dose level were further treated with the same dose of 2,3,7,8-TCDD administered to mothers on day 4, 11, and 18. Additional groups of suckling rats were exposed to 2,3,7,8-TCDD postnatally by oral treatment of mothers with 5 ug/kg. Reduction in body, thymus, spleen, and adrenal weights were observed in different groups at 25 days of age. Splenic lymphocytes of rats exposed postnatally to the 5 ug/kg level showed a decrease in phytohemagglutinin (PHA)-induced DNA synthesis. This effect was not observed in animals exposed pre- and postnatally to 1 ug/kg of 2,3,7,8-TCDD. Thymocytes cultured from postnatally exposed male rats to 5 ug/kg of 2,3,7,8-TCDD showed a significant reduction of thymidine incorporation in the presence of PHA. DNA synthesis in response to concanavalin A (Con-A), however, was not reduced in thymocytes. The authors (Vos and Moore, 1974) reported the insensitivity of 4-month-old mice to 2,3,7,8-TCDD treatment. One-month-old mice treated with four weekly doses of 25 ug/kg of 2,3,7,8-TCDD showed a decreased responsiveness of their splenic lymphocytes to PHA, whereas 5-month-old animals failed to show this effect after six weekly doses.

Similar effects were observed on GVH activity of spleen cells from 25-day-old pre- and/or postnatally exposed rats. Only those animals treated with 5 ug/kg postnatally showed a significant decrease in GVH activity of spleen cells. Reaction times of heterologous skin grafts was prolonged in rats exposed in

utero and in mice exposed pre- and postnatally to 2,3,7,8-TCDD (Vos and Moore, 1974).

In summary, exposure of mice, rats, and/or guinea pigs (as described earlier) to 2,3,7,8-TCDD during the perinatal period resulted in thymic atrophy (Faith and Moore, 1977; Vos and Moore, 1974; Luster et al., 1980; Moore and Faith, 1976; Faith et al., 1978). These animals exhibited a "wasting syndrome" that is similar to that seen in neonatally thymectomized animals that have been treated with corticosteroids (Thomas and Faith, 1985). 2,3,7,8-TCDD administered during immune ontogenesis has been observed to affect immune responses more dramatically than when administered to adults. Generally, thymic atrophy, suppressed T-cell mediated responses, and bone marrow toxicity have been reported to be more profound following pre- and/or postnatal exposure to 2,3,7,8-TCDD than with adult exposure (Dean and Lauer, 1984; Dean and Kimbrough, 1986; Thomas and Faith, 1985; Luster et al., 1979; 1980; Faith and Moore, 1977; Vos and Moore, 1974). These results suggest that the developing immune system is more susceptible to 2,3,7,8-TCDD-induced alterations and, consequently, that the very young may be at a higher risk than adults to the immunotoxic effects of 2,3,7,8-TCDD.

Exposure of animals to 2,3,7,8-TCDD has been shown to decrease the responsiveness of lymphocytes to various mitogens in culture. For example, rabbits exposed for 8 weeks to 2,3,7,8-TCDD at 0.01 to 10 ug/kg/week and challenged with tetanus toxoid had a decreased PHA response at the highest dose (Sharma et al., 1979). Sharma et al. (1979) also reported that immunologic effects of a single dose of 2,3,7,8-TCDD in mice were reversible during an 8-week period while the hepatic lesions persisted. Mice were treated with a single oral dose of 10 ug/kg of 2,3,7,8-TCDD, and selected animals were sacrificed at 2, 4, and 8 weeks after this dosing. The spontaneous increase of DNA synthesis in

splenic cultures and a decreased responsiveness of splenic lymphocytes to phytohemagglutinin and pokeweed mitogen was apparent at 2 weeks after the treatment. The effects persisted up to 4 weeks after the administration of 2,3,7,8-TCDD, but were not noticed when the spleens were obtained from animals at 8 weeks after the 2,3,7,8-TCDD dosing. Lymphocyte depletion in the thymus and reduced thymus weights showed a partial recovery at this time.

The influence of direct addition of 2,3,7,8-TCDD to mouse-splenic cultures was reported by Sharma and Gehring (1979). 2,3,7,8-TCDD decreased the unstimulated DNA synthesis in lymphocytes at concentrations as low as 10^{-9} M; however, no effects were observed in mitogen-stimulated cultures. Vos and Moore (1974) reported that the addition of 2,3,7,8-TCDD up to 0.01 ug/0.5 mL in culture medium did not alter the DNA synthesis in mouse spleen or rat thymus cells, either with or without the presence of phytohemagglutinin or Con-A. Luster and co-workers (1979) exposed the spleens from mice to 2,3,7,8-TCDD in dimethylsulfoxide. DNA, RNA, and protein synthesis in the spleens were inhibited at 2,3,7,8-TCDD concentrations of 10^{-7} M. The ability of lymphocytes to bind with mitogens was not influenced by 2,3,7,8-TCDD.

The mechanism(s) for 2,3,7,8-TCDD-induced immunosuppression is not fully known. However, genetic and structure-activity relationship studies have provided evidence that 2,3,7,8-TCDD-induced immunosuppression in mice is associated with the presence of the Ah locus and is mediated through a 2,3,7,8-TCDD cytosol receptor (Nagarkatti et al., 1984; White et al., 1986; Tucker et al., 1986; Luster et al., 1985; Vecchi et al., 1983; Holsapple et al., 1986; Poland and Glover, 1980; Dencker et al., 1985; Kouri and Ratrie, 1975). Some evidence suggests that thymic epithelial cells may be the principal target for 2,3,7,8-TCDD-induced immunotoxicity (Clark et al., 1983; Greenlee et al., 1985). Binding of 2,3,7,8-TCDD to receptors in the thymus may promote altered T-cell matu-

ration and differentiation, and it may be the molecular basis for the observed thymic atrophy and immunotoxicity. Other work, however, suggests that hematopoietic stem cells and B-cells may also be targets for 2,3,7,8-TCDD-mediated immune effects. For example, it has recently been shown that 2,3,7,8-TCDD selectively inhibits the differentiation of B-cells into antibody-secreting cells in vitro (Tucker et al., 1986) and inhibits bone marrow stem cell colony growth in vivo and in vitro (Luster et al., 1985). The binding of 2,3,7,8-TCDD to receptors on lymphocytes, thymus epithelial cells, and/or hematopoietic precursor cells may cause alterations in maturation and differentiation that may result in the immune alterations observed in animals following in vivo exposure to 2,3,7,8-TCDD. Further work is clearly needed to investigate the mechanism(s) for 2,3,7,8-TCDD-induced immune effects in order to make better estimates of the potential risks associated with exposure of humans to 2,3,7,8-TCDD.

HUMAN STUDIES

For a variety of reasons, humans have been exposed to 2,3,7,8-TCDD in the environment. The immune function has been examined in individuals with probable or known exposure to 2,3,7,8-TCDD using assays designed to evaluate the component parts of the immune system. Several accounts of immune functions in humans exposed to 2,3,7,8-TCDD have been referred to in summary-type articles reviewed in the preparation of this paper. Some of these reports have not been published in the scientific literature or are anecdotal. However, none of these reports, in the opinion of the reviewers (Dean and Lauer, 1984; Dean and Kimbrough, 1986; Marshall, 1986), presented convincing evidence for altered immune function in the exposed populations. In one study, no abnormalities in measured immune parameters were observed in military personnel who had been

involved in the spraying of 2,3,7,8-TCDD-contaminated Agent Orange during operation Ranch Hand in Vietnam (conversation between J. Silva, CHHS, Test and Evaluation Activity, Bethesda, MD, and Dr. Ralph Smialowicz, U.S. Environmental Protection Agency, February 6, 1987).

Attempts have been made to study immune functions in populations that have been exposed to 2,3,7,8-TCDD. All of these attempts have been complicated by technical difficulties in both design and execution, in spite of efforts by the researchers to control for all possible variables. Three such studies are summarized.

In July of 1976, an uncontrolled chemical reaction at a chemical plant in Seveso, Italy, resulted in the release of an estimated 300 g of 2,3,7,8-TCDD mainly into an uninhabited area. However, a significant number of people were potentially exposed, and thus, a major health effects surveillance effort was initiated. The results of this study, including immunologic assessment, have been published (Homberger et al., 1979; Reggiani, 1980). A group of 45 2,3,7,8-TCDD-exposed children, 20 of whom had chloracne, and 44 children without 2,3,7,8-TCDD exposure were evaluated immunologically every 4 months for approximately 1.5 years. These studies revealed no differences between the two groups in serum immunoglobulin or complement levels or in the ability of their T- and B-cells to respond to mitogens in vitro. Critical evaluation of these data are not possible, however, since quantitative data were not presented. It should be noted that a review (Tognoni and Bonaccorsi, 1982) of the Seveso incident, published 5 years after the fact, reported increased serum complement hemolytic activity and significantly higher lymphocyte proliferative responses in exposed subjects. However, no quantitative data were presented, the patient population was not identified per se, and no reference was made as to how or when these data were collected. Thus, critical review and interpretation of the reported

findings are both impossible and unadvisable.

The town of Times Beach, Missouri, was contaminated with 2,3,7,8-TCDD in 1972 and 1973 when waste from a chemical plant was sprayed on roadways to control dust. Soil samples were tested for 2,3,7,8-TCDD 10 years later, and levels of contamination were so great that the entire town was purchased under the provisions of the Superfund law (Powell, 1984). Selected residents were subsequently classified as having had high or low risk of 2,3,7,8-TCDD exposure and were evaluated immunologically (Knutsen, 1984). Tests of delayed type hypersensitivity (DTH) to a standard battery of skin test antigens, lymphocyte blastogenic responses to mitogens, and T-cell subset analysis revealed no significant differences between the high- and low-risk groups. However, there was a tendency among members of the high-risk group to respond to fewer skin test antigens and to have slightly different T-cell subset profiles than low-risk group members. Lymphocytes from children in the high-risk group also had a decreased proliferative response to tetanus toxoid compared with those from children in the low-risk group. It should be noted, however, that in a preliminary study of an unspecified population of Missouri residents exposed to 2,3,7,8-TCDD, no differences were detected in T-cell subset profiles, skin test responses, or lymphocyte proliferation responses (Stehr et al., 1986).

Residents of the Quail Run Mobile Home Park in Gray Summit, Missouri, were exposed for various lengths of time to 2,3,7,8-TCDD-contaminated soil. Contamination was the result of dust control efforts using waste oil containing chemical sludge from the same source as in Times Beach. Soil sampling studies revealed levels of 2,3,7,8-TCDD ranging from 39 to 2,200 parts per billion. Immunologic testing was performed on residents who had lived in the park for at least 6 months (N = 154), and their results were compared to residents (N = 155) of other mobile parks in areas where no evidence of 2,3,7,8-TCDD

contamination was found by soil sampling (Hoffman et al., 1986). DTH to skin test antigens, antigen- and mitogen-stimulated lymphocyte proliferation responses, lymphocyte subset analysis, cytotoxic T-lymphocyte responses, and serum immunoglobulin levels were evaluated in both populations. Some members of the high-risk group did not respond to skin test antigens (anergy) and, of those that did respond, positive reactions were obtained for fewer antigens than in the unexposed group (relative anergy). The exposed group had an increased frequency of anergy (11.8% vs. 1.1%) and relative anergy (35.3% vs. 11.8%). It must be pointed out that there were significant technical problems with the interpretation of the skin test responses and, as a result, nearly 50% of the data had to be discarded. Several skin test readers, with various amounts of experience, were employed in this investigation. In addition, two of the four readers who had received special training in the interpretation of DTH skin tests recorded anergy in 15% or 40% of the control group, a rate 75 or 200 times the expected rate. Although allowances were made for this by the investigators, the DTH data in this study are thus questionable. The mean ratio of T helper/inducer cells to T suppressor/cytotoxic cells was similar in both groups, although there was a nonsignificant proportion of exposed group members with a T4/T8 ratio less than 1.0 (8.1% vs. 6.4%). The report likewise states that 12.6% of the exposed group versus 8.5% of the low-risk group had abnormal in vitro T-cell functions, although examination of the tabular data provides no evidence whatsoever for a difference between levels of immunocompetence in the two groups. The authors concluded that long-term exposure to 2,3,7,8-TCDD is associated with depressed cell-mediated immunity, although the effects have not resulted in an excess of clinical illness. Individuals from this initial study (Hoffman et al., 1986) have been re-evaluated, and the results of this follow-up study, as reported by Evans et al. (1987) at the

International Conference on Dioxin, failed to corroborate the report of anergy in the 2,3,7,8-TCDD exposed cohort of the initial study (Hoffman et al., 1986).

UNCERTAINTIES, DATA GAPS, AND RESEARCH NEEDS

The immune system, unlike many organ systems, is self-renewing from a pool of pluripotent stem cells. Functional cells are generally end-stage cells that have a limited lifetime and are replaced on a regular basis. Therefore, unless the store of stem cells is destroyed or unless there is a permanent blockade of cellular differentiation, acute chemical exposure is unlikely to cause long-term suppression of the host-defense system. However, the uncertainty in the premise of self-limited chemical immunotoxicity resides in the unknown effects of chronic or repeated acute exposure to immunotoxic agents on the regenerative capacity of the immune system.

Although a great amount of time and effort has gone into evaluating the immunotoxic effects of 2,3,7,8-TCDD in experimental animals, there are a number of uncertainties that remain to be resolved. These include but are not limited to the following: (1) the lack of a strong association between 2,3,7,8-TCDD exposure and decreased host resistance, (2) the different susceptibility of species and strains to 2,3,7,8-TCDD-induced immunosuppression, (3) the transient and reversible nature of 2,3,7,8-TCDD-induced immune effects, (4) the apparent but not proven increased susceptibility of very young animals to 2,3,7,8-TCDD-induced immune effects, (5) the uncertainty of the mechanism(s) of 2,3,7,8-TCDD-induced immune effects, and (6) the lack of evidence between observed immunological effects in animals and the questionable immune alterations reported in human populations inadvertantly exposed to 2,3,7,8-TCDD. These areas of uncertainty are briefly discussed.

Inconsistent results have been reported regarding the susceptibility of 2,3,7,8-TCDD-exposed animals to tumor development and infection (Dean and Lauer, 1984; Faith and Moore, 1977; Luster et al., 1980; Thigpen et al., 1975; White et al., 1986; Tucker et al., 1986). Despite the fact that exposure to 2,3,7,8-TCDD results in depressed T- and B-cell responses, further research is necessary to define the conditions under which host resistance is adversely affected by 2,3,7,8-TCDD.

In general, the experimental animal data suggest that species differences exist in 2,3,7,8-TCDD sensitivity. With the exception of the earliest work, the mouse has been the predominant species for studying the immunotoxic effects of 2,3,7,8-TCDD. This is most probably due to the fact that more is known about the immune system of the mouse than any other animal species, as well as the fact that there are more validated methods available for examining the immune system in this species. Nevertheless, extensive interspecies comparisons among several animal species are warranted considering their pharmacokinetic differences. These studies are needed not only to substantiate and corroborate the results of studies with the mouse but also to provide the framework to extrapolate the potential for 2,3,7,8-TCDD to affect the human immune system. Interspecies studies are also necessary in order to determine if an association exists between the Ah locus and 2,3,7,8-TCDD-induced immune effects in species other than the mouse. This includes extension of in vitro studies which have demonstrated the presence and inducibility of AHH in human lymphoid tissue (Kouri and Ratrie, 1975). Hopefully, these studies will provide useful information about the role that the Ah locus may play in the susceptibility of the human population to 2,3,7,8-TCDD.

The immune effects that have been observed following 2,3,7,8-TCDD exposure have, in all cases where it has been examined, returned to normal levels over

a period of time after the cessation of exposure to 2,3,7,8-TCDD. This is probably a result of the plastic nature of the immune system, as well as the fact that chronic dosing with 2,3,7,8-TCDD has not been performed. It is not clear whether and how repeated doses might affect the immune system or whether short-term exposure could result in irreversible effects. Low-dose chronic studies in animals are needed to determine whether such exposures not only produce immune alterations but also to determine if long-lasting impairment is produced. Low-dose chronic studies are important to extrapolate data from the high dosages used under experimental situations because they are most likely to mimic the form of human exposure to 2,3,7,8-TCDD in the environment.

Exposure to 2,3,7,8-TCDD during immune system development has been shown to affect the immune system of experimental animals (Faith and Moore, 1977; Vos and Moore, 1974; Luster et al., 1980; Faith et al., 1978). The effects produced following perinatal exposure have been reported to be more profound than those produced following adult exposure, although in some cases a high degree of fetal toxicity has been reported (Vos and Moore, 1974). While this may be true, no attempt has been made to test this hypothesis by making a direct comparison between perinatal and adult exposure to 2,3,7,8-TCDD using identical exposure regimens (i.e., B- and T-cell function, host resistance models, natural killer cell activity, etc.). Work is warranted in this area in order to provide evidence of an increased risk of the developing immune system to 2,3,7,8-TCDD exposure. This is necessary so that an informed judgment can be made as to the potential increased relative risk to infants and children exposed to 2,3,7,8-TCDD.

There is still a great deal of uncertainty about the mechanism(s) by which 2,3,7,8-TCDD causes immune alterations in animals. Work with other animal species and in vitro work with human tissues will hopefully provide new insights

in this area. A clearer understanding of the mechanisms of 2,3,7,8-TCDD-induced immunosuppression in animals will be invaluable for extrapolation of the potential health risk in humans.

Laboratory studies have clearly demonstrated the immunotoxic effects of 2,3,7,8-TCDD in a variety of animal models. Extrapolation of these data to predict effects of human 2,3,7,8-TCDD exposure are complicated at best, since actual exposure levels, route of exposure, and even comparability of human and animal susceptibility to the toxic effects of 2,3,7,8-TCDD are unknown. Results of rodent studies suggest that the immune responses of children should be more sensitive to the immunotoxic effects of 2,3,7,8-TCDD than that of adults (Faith and Moore, 1978). However, immune function was followed in children from the most heavily contaminated area of Seveso for 18 months after the accident and appeared to be normal (Homberger et al., 1979; Reggiani, 1980). Furthermore, baseline data for the immune system of children is not readily available, and the normal response in children of various ages is not well defined. Furthermore, altered immunocompetence has been reported in 2,3,7,8-TCDD-exposed residents of Missouri (Knutsen 1984; Hoffman et al., 1986), although the differences between mean values for control and exposed populations were not statistically significant. Suppression was not detected in functional parameters; rather, trends in the distribution of exposed group members into "normal" and "abnormal" response categories were cited as indicative of immune dysfunction. While these trends may or may not be related to 2,3,7,8-TCDD exposure, there has been no report of an increase in clinical illness attributable to suppressed immune function. Thus, it appears to be that there are no unequivocal cases of significant immunotoxicity in humans following 2,3,7,8-TCDD exposure (Dean and Lauer, 1984; Dean and Kimbrough, 1986; Marshall, 1986; Evans et al., 1987).

The available animal data reviewed above suggest that 2,3,7,8-TCDD-induced thymus atrophy and immune alterations may result from direct actions on peripheral lymphocytes or progenitor lymphoid cells in the bone marrow and through altered differentiation of intrathymic precursor cells, specifically by a direct action on thymic epithelium. The induction of T-cells, cytotoxic for tumor target cells, was found to be impaired in mouse studies conducted by Clark et al. (1981) following total exposure to a 2,3,7,8-TCDD dose of 4 ng/kg over a 4-week period. These dosages are below those levels significantly altering other cell-mediated immune responses (Clark et al., 1981); however, these results are unconfirmed and questionable. For example, the kinetics of CTL suppression was not defined in the Clark et al. studies (1981, 1983), and the effect of 2,3,7,8-TCDD on CTL-mediated tumor resistance remains to be resolved. Additional concern is raised about Clark's findings (Clark et al., 1981) by researchers at the Chemical Industry Institute of Toxicology (CIIT) who claim that the CTL response was not suppressed at a dose of 4 ng/kg (conversation between Jack Dean, CIIT, Research Triangle Park, NC, and Bob Sonawane, U.S. Environmental Protection Agency, February 17, 1987). The ED_{50} (dose producing 50% maximal response) for the induction of thymic atrophy in sensitive mouse strains is approximately 10 umol/kg (Poland and Glover, 1980) and for antibody plaque-forming cell (PFC) suppression varies by 30-fold (1 ug/kg to 30 ug/kg) between C57BL/6 and DBA/2 mice (Dean and Lauer, 1984). Furthermore, the issue of host-resistance effects following exposure to 2,3,7,8-TCDD is unresolved.

In summary, it may be inappropriate to derive an immunotoxicity-based hazard assessment for 2,3,7,8-TCDD from mostly acute and/or subchronic type studies. A three-generation reproductive study by Murray et al. (1979) demonstrated the critical noncancer end point for adverse effects at 1 ng/kg/day compared to questionable immunosuppressive effects observed in mice by Clark

et al. (1981) at 4 ng/kg by parental administration. It seems that the level of concern identified in either of these studies or in chronic bioassays for carcinogenicity is essentially the same. The magnitude of differences reinforces a common conclusion that the biological significance of any immunological effects observed in laboratory animals is not adequately established to support its use as the critical end point in hazard evaluation of 2,3,7,8-TCDD exposure to humans.

REFERENCES

Chastain, J.E.; Pazdernik, T.L. (1985) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced immunotoxicity. Int. J. Immunopharmacol. 7:849-856.

Clark, D.A.; Gauldie, J.; Szewczuk, M.R.; Sweeney, G. (1981) Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachloro-dibenzo-p-dioxin. Proc. Soc. Exp. Biol. Med. 168:290-299.

Clark, D.A.; Sweeney, G.; Safe, S.; Hancock, E.; Kilburn, D.G.; Gauldie, J. (1983) Cellular and genetic basis for suppression of cytotoxic T-cell generation by haloaromatic hydrocarbons. Immunopharmacology 6:143-153.

Dean, J.H.; Kimbrough, R.D. (1986) Immunotoxicity of the chlorinated dibenzo-p-dioxins and dibenzofurans. Presented at the Dioxin Update Conference; July 1-2; Alexandria, VA. Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency.

Dean, J.H.; Lauer, L.D. (1984) Immunological effects following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin: a review. In: Lowrence, W.W., ed. Public health risk of the dioxins. Los Altos, CA: W. Kaufmann, pp. 275-294.

Dencker, L.; Hassoun, E.; Dar-gy, R.; Alm, G. (1985) Fetal thymus organ culture as an in vitro model for the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and its congeners. Mol. Pharmacol. 27:133-140.

Evans, R.G.; Webb, K.B.; Knutsen, A.P.; Roodman, S.T.; Roberts, D.; Bagby, J.; Garrett, W.A., Jr.; Andrews, J.S. (1987) A medical follow-up of the health effects of long-term exposure to 2,3,7,8-TCDD. 7th International Conference on Dioxin; October; Las Vegas, NV, pp. EP08.

Faith, R.E.; Moore, J.A. (1977) Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). J. Toxicol. Environ. Health 3:451-464.

Faith, R.E.; Luster, M.I.; Moore, J.A. (1978) Chemical separation of helper cell function and delayed hypersensitivity responses. Cell Immunol. 40:275-284.

Greenlee, W.F.; Dold, K.M.; Irons, R.D.; Osborne, R. (1985) Evidence of direct action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on thymic epithelium. Toxicol. Appl. Pharmacol. 79:112-120.

Gupta, B.N.; Vos, J.G.; Moore, J.A.; Zinkl, J.G.; Bullock, B.C. (1973) Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Perspect. 5:125-140.

Harris, H.W.; Moore, J.A.; Vos, J.G.; Gupta, B.N. (1973) General biological effects of TCDD in laboratory animals. Environ. Health Perspect. 5:101-109.

Hoffman, R.E.; Stehr-Green, P.A.; Webb, K.B.; Evans, G.; Knutsen, A.P.; Schramm, W.F.; Staake, J.L.; Gibson, B.A.; Steinberg, K.K. (1986) Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. JAMA 255:2031-2038.

Holsapple, M.P.; McCay, J.A.; Barnes, D.E. (1986) Immunosuppression without liver induction by subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in adult female B6C3F1 mice. Toxicol. Appl. Pharmacol. 83:445-455.

Homberger, E.; Reggiani, G.; Sambeth, J.; Wipf, H.K. (1979) The Seveso accident: its nature, extent, and consequences. Ann. Occup. Hyg. 22:327-367.

Knutsen, A.P. (1984) Immunologic effects of TCDD exposure in humans. Bull. Environ. Health 6:27-43.

Kouri, R.E.; Ratrie, H., III; Atlas, S.A.; Niwa, A.; Nebert, D.W. (1975) Aryl hydrocarbon hydroxylase induction in human lymphocyte cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Life Sci. 15:1585-1595.

Luster, M.I.; Faith, R.E.; Clark, G. (1979) Laboratory studies on the immune effects of halogenated aromatics. Ann. N.Y. Acad. Sci. 320:473-486.

Luster, M.I.; Boorman, G.A.; Dean, J.H.; Harris, M.W.; Luebke, R.W.; Padarathsingh, M.L.; Moore, J.A. (1980) Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Int. J. Immunopharmacol. 2:301-310.

Luster, M.I.; Dean, J.H.; Boorman, G.A. (1982) Altered immune functions in rodents treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin, phorbal-12-myristate-13-acetate, and benzo(a)pyrene. In: Hunt, V.R.; Smith, M.K.; Worth, D., eds. Environmental factors in human growth and development. Banbury Report No. 11. Banbury, NY: Cold Spring Harbor Laboratory, pp. 199-215.

Luster, M.I.; Hong, L.H.; Boorman, G.A.; Clark, G.; Hayes, H.T.; Greenlee, W.F.; Dold, K.; Tucker, A.N. (1985) Acute myelotoxic responses in mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 81:156-165.

Mantovani, A.; Vecchi, A.; Luini, W.; Sironi, M.; Candiani, G.P.; Spreafico, F.; Garattini, S. (1980) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on macrophage and natural killer cell-mediated cytotoxicity in mice. Biomedicine 32(4):200-204.

Marshall, E. (1986) Immune system theories on trial. Science 234:1490-1492.

Moore, J.A.; Faith, R.E. (1976) Immunologic response and factors affecting its assessment. Environ. Health Perspect. 18:125-131.

Murray, E.J.; Smith, F.A.; Nitschke, K.D.; Humiston, C.G.; Kociba, R.J.; Schwetz, B.A. (1979) Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50:241-251.

Nagarkatti, P.S.; Sweeney, G.D.; Gauldie, J.; Clark, D.A. (1984) Sensitivity to suppression of cytotoxic T-cell generation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is dependent on the Ah genotype of the murine host. Toxicol. Appl. Pharmacol. 72:169-176.

Pazdernik, T.L.; Rozman, K.K. (1984) Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced immunotoxicity. Life Sci. 36:695-703.

Poland, A.; Glover, E. (1980) 2,3,7,8-tetrachlorodibenzo-p-dioxin: segregation of toxicity with the Ah locus. Mol. Pharmacol. 17:86-94.

Powell, R.L. (1984) Dioxin in Missouri: 1971-1983. Bull. Environ. Contam. Toxicol. 33:648-654.

Reggiani, G. (1980) Acute human exposure to TCDD in Seveso, Italy. J. Toxicol. Environ. Health 6:27-43.

Sharma, R.P.; Gehring, P.J. (1979) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on splenic lymphocyte transformation in mice after single and repeated exposures. Ann. N.Y. Acad. Sci. 320:487.

Sharma, R.P.; Kociba, R.J.; Gehring, P.J. (1979) Reversal of immunologic and toxicologic effects of a single exposure of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice. Trace Substances Environ. Health 12:473.

Stehr, P.A.; Stein, G.; Falke, H.; Sampson, E.; Smith, S.J.; Steinberg, K.; Webb, K.; Ayres, S.; Schramm, W.; Donnell, H.D.; Gedney, W.B. (1986) A pilot epidemiological study of possible health effects associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin contaminants in Missouri. Arch. Environ. Health 41:16-22.

Thigpen, J.E.; Faith, R.E.; McConnel, E.E.; Moore, J.A. (1975) Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Infect. Immun. 12:1319-1324.

Thomas, P.T.; Faith, R.E. (1985) Adult and perinatal immunotoxicity induced by halogenated aromatic hydrocarbons. In: Dean, J.; Luster, M.; Munson, A.; Amos, H.E., eds. Toxicology of the immune system. New York, NY: Raven Press, pp. 305-313.

Tognoni, G.; Bonaccorsi, A. (1982) Epidemiological problems with TCDD: a critical review. Drug Metab. Rev. 13:447-469.

Tucker, A.N.; Vore, S.J.; Luster, M.I. (1986) Suppression of B cell differentiation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol. Pharmacol. 29:372-377.

U.S. Environmental Protection Agency (1985) Health assessment document for polychlorinated dibenzo-p-dioxins. Office of Health and Environmental Assessment, Washington, DC. EPA/600/8-84/014F. NTIS PB86-122546/AS.

van Logten, M.J.; Gupta, B.N.; McConnel, E.E.; Moore, J.A. (1980) Role of the endocrine system in the action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the thymus. Toxicology 15(2):135-144.

Vecchi, A.M.; Sironi, M.; Canegrati, M.A.; Recchia, M.; Garattini, S. (1983) Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. Toxicol. Appl. Pharmacol. 68:434-441.

Vos, J.G.; Moore, J.A. (1974) Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int. Arch. Allergy Appl. Immunol. 47:777-790.

Vos, J.G.; Moore, J.A.; Zinkl, J.G. (1973) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ. Health Perspect. 5:149-162.

Vos, J.G.; Moore, J.A.; Zinkl, J.G. (1974) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57BL/6 mice. Toxicol. Appl. Pharmacol. 29:229-241.

Vos, J.G.; Kreeftenberg, J.G.; Engel, H.W.; Miderhoud, A.; van Noorle, J.L.M. (1978) Studies on 2,3,7,8-tetrachlorodibenzo-p-dioxin induced immune suppression and decreased resistance to infection: endotoxin hypersensitivity, serum zinc concentrations and effect of thymosin treatment. Toxicology 9(1-2):75-86.

White, K.L.; Lysy, H.H.; McCay, A.; Anderson, A.C. (1986) Modulation of serum complement levels following exposure to polychlorinated dibenzo-p-dioxins. Toxicol. Appl. Pharmacol. 84:209-219.

Zinkl, J.G.; Vos, J.G.; Moore, J.A.; Gupta, B.N. (1973) Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Health Perspect. 5:111-118.

June 1988
Review Draft

APPENDIX F

RATIONALE FOR A HORMONE-LIKE MECHANISM OF 2,3,7,8-TCDD FOR USE IN RISK ASSESSMENT

Michael A. Gallo
Professor and Chief, Division of Toxicology
Department of Environmental and Commmunity Medicine
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School
675 Hoes Lane
Piscataway, NJ 08854-5635

Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

The mechanisms of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) have been intensely investigated since the pioneering work of Kimmig and Schultz (1957) in elucidating the chloracnegen in the chlorophenol processes. The most complete and thought-provoking treatise on the subject is that of Poland and Knutson (1982). These authors and others in Poland's laboratory drew upon their work and others to present a unified hypothesis to account for the varied responses in animals exposed to 2,3,7,8-TCDD. In essence, this hypothesis, which is partly based on the classic receptor theories invoked for steroid action, suggests that (1) there is a cytosolic receptor for arylhydrocarbons (the Ah receptor) that binds several compounds and then translocates to the nucleus, and (2) there is a second stage to the toxic reaction(s) that is related to, but not congruent with, the induction of cytochrome P_1-450. Activation of this receptor leads to a cascade of reactions culminating with the association of the receptor-2,3,7,8-TCDD complex with nuclear DNA. This association leads to the synthesis of a specific microsomal protein designated as cytochrome P_1-450. To date, the chemical that binds this putative receptor with the greatest avidity is 2,3,7,8-TCDD. However, many other halogenated and non-halogenated compounds also bind to this cytosolic protein (Nebert et al., 1972). The xenobiotics with the greatest affinity are those that are planar, with two phenyl rings and contain substitutions in the lateral positions. Several investigative teams have examined the structure activity relationships (SARs) among the polyhalogenated biphenyls (PCBs, PBBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) (Knutson and Poland, 1980). Safe and his co-workers have synthesized several of the highly active PCBs and have demonstrated remarkable SARs for several biological end points (Mason et al., 1987). Excellent reviews on the

SAR for PCDDs, PCDFs, and PCBs can be found in recent issues of the Annual Reviews in Pharmacology and Toxicology (Vols. 22 [Poland and Knutson, 1982] and 26 [Safe, 1986], respectively). The hypothesis also states that ". . .there is a second stage to the toxic reactions of 2,3,7,8-TCDD that are related to, but not congruent with, the induction of cytochrome P_1-450." This portion of the hypothesis is supported by the reports of Poland and his co-workers with XB cells in culture (Knutson and Poland, 1980), Safe and co-workers' findings of PCB inhibition of 2,3,7,8-TCDD toxicity (Haake et al., 1987), and the Umbreit et al. report of apparent maximal induction of arylhydrocarbon hydroxylase (the major system affected by cytochrome P_1-450) (Umbreit et al., 1987) by complex mixtures of polyaromatic hydrocarbons and very low bioavailability of PCDDs/PCDFs as measured by tissue levels and signs of toxicity.

At this point in time, it appears clear that 2,3,7,8-TCDD is working through the proposed receptor mechanism for the first phase of its activity (i.e., binding to a putative cytosolic receptor with subsequent induction of P-450). However, all of the biological effects of this molecule cannot be explained by simple receptor binding and induction of cytochrome P_1-450. The recent evidence from several laboratories has expanded on the initial studies of Poland and Knutson (1982), Neal et al. (1982), and Barsotti et al. (1979) to show quite dramatically that 2,3,7,8-TCDD markedly affects the interaction of steroids with their respective receptors (Romkes et al., 1987; Gallo et al., 1986) and 2,3,7,8-TCDD alters the number of Epidermal Growth Factor (EGF) receptors in susceptible cell lines (Matsumura et al., 1984). Molloy et al. (1987) recently reported the alteration of specific epidermal keratins in the HRJ/S strain of mice after treatment with 2,3,7,8-TCDD. This finding is especially relevant to the database since it was in this strain of mice that

Poland and Knutson (1982) reported the model for chloracne. Matsumura et al. (1984) studied the role of 2,3,7,8-TCDD and EGF receptors, while several laboratories (Safe, 1986; Gierthy et al., 1987; Umbreit and Gallo, 1988; Goldstein et al., 1987) have been pursuing the interactions of 2,3,7,8-TCDD with steroids, primarily estrogen-sensitive tissues. The interactions with glucocorticoids has been studied extensively by several laboratories (Luster et al., 1984; Sunihara et al., 1987). In general, the response can be summarized as a decrease in the number of available cytosolic receptors for EGF or the steroids without a decrease in the affinity for the respective ligand. This phenomenon is termed "down-regulation" of the cytosolic receptor. The measurement of receptor binding is biochemical. The estimate of affinity and binding site number is by extrapolation of the response curve(s) by Scatchard analysis (1949). The strengths of this analysis are obvious, but the weaknesses are difficult to reconcile. The major weakness is the lack of direct binding information; this does not allow segregation of the receptors by tertiary structure nor does it completely account for nonspecific binding. The second weakness of ligand binding experiments is the inability of the analysis to shed any light on the reason for the down-regulation. It must be emphasized at this point that none of the steroid receptor research has demonstrated an antagonism between 2,3,7,8-TCDD and the endogenous steroid for the respective steroid receptor, nor has any competitive binding of steroids by the Ah receptor been demonstrated. However, the steroid receptors are products of a supergene family which is responsible for the protein synthesis of all these receptors (Nebert et al., 1972), and the Ah receptor has many of the structural and functional characteristics of the steroid receptors (Poellinger et al., 1986).

To better understand the role of 2,3,7,8-TCDD in cellular function (or dysfunction), one must look to the results of the laboratories working on the mechanisms of action of 2,3,7,8-TCDD at the molecular level. The major groups involved in this research are Poland, Nebert's group at the National Institutes of Health, and Whitlock's laboratory at Stanford University. As stated above, Poland established the role of the Ah receptor in some of the actions of 2,3,7,8-TCDD. Whitlock (1987) has summarized his data and that of other investigators regarding the regulation of the cytochrome P-450 gene family, along with the data supporting the hypothesis that the Ah locus is part of a super gene family responsible for the metabolism of xenobiotics and endogenous compounds. Recent work in this laboratory has also elucidated a region on DNA, which is sensitive to the 2,3,7,8-TCDD-cytosolic receptor complex, upstream from the cytochrome P_1-450 gene (Neuhold et al., 1986). These findings are critical in light of the findings of the 2,3,7,8-TCDD- responsive gene expression enhancer system (region) described by Whitlock (Jones et al., 1986). Hence, the two laboratories have defined the regulatory mechanisms by which 2,3,7,8-TCDD controls gene expression of the cytochrome P_1-450 (Whitlock, 1987). The significance of these findings for 2,3,7,8-TCDD risk analysis is the congruency between gene regulation for the Ah receptor, the glucocorticoid receptor, and the estrogen receptor (Becker et al., 1986). The importance of these findings cannot be underestimated. There is direct analogy with the steroid receptor mechanisms and the control of the steroid receptor messenger RNA (Yamamoto, 1985). The role of the estrogen receptor (ER), and other steroid receptors, is understood to a greater extent than the Ah receptor probably because of the greater emphasis on the physiology of steroids. The analogy between the receptor complexes and DNA leads to the obvious comparison

of effects of 2,3,7,8-TCDD and steroids. Many of the changes seen in animals after dosing with 2,3,7,8-TCDD mimic estrogen or antiestrogen effects. Umbreit and Gallo (1988) reviewed these findings, which are presented in Table 1. Kociba et al. (1978) demonstrated the hepatocarcinogenic effect of orally administered 2,3,7,8-TCDD, but in the same study there was a marked dose-dependent decrease in tumors of the mammary glands and uteri which are estrogen-sensitive organs. These are highly significant observations which have been pursued by some laboratories. If 2,3,7,8-TCDD is acting through hormonal (estrogen) mechanisms, then alteration of ovarian function, exogenous estrogens, or antiestrogens should modify the response(s) to 2,3,7,8-TCDD. Recent results have shown that 2,3,7,8-TCDD effects can be overridden by exogenous estradiol (Gallo et al., 1986) and the down-regulation of the estrogen receptor is also antagonized by estradiol (Romkes et al., 1987). The significance of these findings are amplified if one couples the reports of regulation of the EGF receptor by estrogens (Mukku and Stancel, 1985; Madhukar et al., 1984) along with the consistent observation that the lowest doses in the lifetime bioassays of 2,3,7,8-TCDD decrease tumor yield in rodent livers but do not affect the backround levels of breast or uterine tumors (Kociba et al., 1978). 2,3,7,8-TCDD inhibition of tumor growth at low doses and enhancement at higher levels (in the bioassays) is supported by the recent report of a marked decrease in tumorigenesis in the two-stage liver model at the lowest dose of 2,3,7,8-TCDD after diethylnitrosamine (DEN) initiation (Pitot et al., 1987). These findings are consistent with the hypothesis that 2,3,7,8-TCDD may be working through an endocrine-sensitive mechanism to yield its toxic effects. If one accepts this premise, then it is reasonable to assume that the actions of 2,3,7,8-TCDD can be explained using a

TABLE 1. ASSOCIATION OF EXTROGENS WITH 2,3,7,8-TCDD TOXICITY

Many of the toxic effects of 2,3,7,8-TCDD are similar to effects of estrogens in non-2,3,7,8-TCDD treated animals.

1. Some effects of 2,3,7,8-TCDD resemble effects of elevated estrogens.

2. Other effect of 2,3,7,8-TCDD resemble antiestrogenic effects [most antiestrogens have estrogenic effects at different doses].

3. Some 2,3,7,8-TCDD effects are not straightforward estrogenic or antiestrogenic effects. For some of these, an influence of estrogen on the effect is known.

4. Other signs of 2,3,7,8-TCDD toxicity may be related to cholesterol mobilization for estrogen synthesis.

TABLE 1. (continued)

2,3,7,8-TCDD EFFECTS:

Fat loss 1,4

Wasting 1,4

Changes in serum lipids: 1,3,4
- increased cholesterol
- increased LDL, VLDL

Anorexia 1

Hypophagia 1

Hypoinsulinemia 1

Altered serum fatty acids 1,4

Hypoglycemia 1,4

Lowered O_2 consumption

Membrane damage 1,4

Stimulates differentiation 1
- in certain epithelial cells

Lower serum testosterone 3

Uterine suppression 2

Reproductive failure 1,2,3

Blockage of E2 uterotrophism 1

Terata 1,2,3

Immunosuppression 1

Thymic involution 1

Decreased thymic cellularity

Hirsutism 1

Chloracne 3

Skin keratinization 1

Lowered T4 in serum 3

Increased:

- thyroid weight 3
- serum TSH
- T4 excretion as glucuronide

TABLE 1. (continued)

Lowered serum corticoids 3,4

Blockage of ACTH stimulation of corticosteroid synthesis 3,4

Downregulates:

- EGF receptor 1
- Prolactin receptor 1
- Glucocorticoid receptor 1
- LDL receptor 1
- Estrogen receptor 1

Ascites 1,4

Hepatocyte membrane damage 1,4

Hepatocyte membrane cAMP reduced

Enzyme inductions 3

- AHH (EROD, P-448, P-450c, and d)
- Ornithine decarboxylase
- UDP-GTs
- ALA synthetase
- Others

Anemia

Porphyria cutanea tarda 1

Iron accumulation in liver

Altered iron transport in gut

physiologically based model. The physiological implications of an endocrine mechanism can explain many of the responses seen in animals after exposure to 2,3,7,8-TCDD (see Table 1) since these responses are similar to hyper- and hypo-hormonal states (O'Malley and Buller, 1977; Potter et al., 1986; Jones et al., 1986; Gustafsson et al., 1987). As stated above, the analogy to the estrogen system is arguably the strongest to 2,3,7,8-TCDD effects (both hyperplastic and dysplastic responses in endocrine sensitive organs), and the similarity between the cytosolic receptors and their stabilization by molybdate (Denison et al., 1986), activation of a nuclear site, and the anti-2,3,7,8-TCDD effect of estradiol strengthens the analogy. The effects of estrogens are widespread throughout the body. Some of these effects may not be receptor-mediated, but the majority of the effects are directly attributable to receptor binding. The toxic effects of estrogens have recently been summarized (Umbreit and Gallo, 1988) and include thymic involution and decreased response to septic challenge (Luster et al., 1984; Grossman, 1984), a wasting syndrome characterized by weight loss, hirsutism, and epidermal lesions. As a note, there are recent reports of estrogens enhancing or causing cachexia and wasting which are major effects of 2,3,7,8-TCDD seen in intoxicated animals. The role of estrogens as immunosuppressives is not well understood, but it is hypothesized that the putative suppressant is either an excess of circulating estradiol or perhaps an excess of trophic hormones. Estrogens also play a role in the action of other hormones and trophic factors such as EGF (Kirkland et al., 1981; Gardner et al., 1978; Mukku, 1984; Mukku and Stancel, 1985; Hsueh et al., 1981; Gonzales et al., 1984; Dickson and Lippman, 1987). These findings lead to the conclusion that the multiple effects of TCDD could be mediated through an endocrine mechanism. The weakness of this assumption is that

2,3,7,8-TCDD causes effects that appear similar to both hyperestrogenemia and hypoestrogenemia. It has been hypothesized that this apparent paradox is the result of 2,3,7,8-TCDD or the ligand complex preventing the endogenous substrate from interacting "correctly" with both the active site and a secondary binding site (Umbreit and Gallo, 1988; Umbreit et al., 1988).

Pleiotropism is not an uncommon finding with molecules such as hormones or in this case 2,3,7,8-TCDD. One has only to review the early experiments on multistage mouse skin carcinogenesis of 2,3,7,8-TCDD to see that in some cases it inhibited tumor formation by PAH initiators (DiGiovanni et al., 1977; Berry et al., 1978). It must be emphasized that the responses in multistage models are dependent on time, sequence of administration, dose, and species. Hence, inhibition under some conditions might have been predictable. This is juxtaposed to the two-stage liver model (Pitot et al., 1980) in which it has been shown that orally administered 2,3,7,8-TCDD enhances the tumorigenic action of DEN. However, in subsequent experiments at lower doses of 2,3,7,8-TCDD, a parabolic dose-response curve has been reported in the DEN/2,3,7,8-TCDD initiation-promotion protocol (Pitot et al., 1987). This paradoxical effect is not well understood, but it does not appear to be solely the function of enhanced metabolism, or Ah receptor binding (Mason et al., 1987). Perhaps it is the result of alteration of EGF receptors at low doses (Madhukar et al., 1984) which displays a commonality with several steroid hormone receptors.

The importance of these findings to the approximation of human and animal health risks from exposure to PCDDs and related molecules cannot be overstated. Mathematical modeling of physiological phenomena, especially those related to receptor function, is conducted using the Michaelis-Menten equation (1913) as modified by Clark for the "classical" receptor model (1933). The weight of

evidence for the most prevalent 2,3,7,8-TCDD effects falls into the category of the receptor model (Poland and Knutson, 1982). The recent finding that the hepatocarcinogenesis is related to estrogen levels or to the presence of functional ovaries (Goldstein et al., 1987), and that DEN hepatocarcinogenesis, in partially hepatectomized rats, is first inhibited then promoted by 2,3,7,8-TCDD (Pitot et al., 1980, 1987) indicate that 2,3,7,8-TCDD is not causing its myriad of effects in liver by a simple one-step event such as binding to the Ah receptor and subsequent induction of cytochrome P_1-450. However, operationally 2,3,7,8-TCDD is a potent hepatocarcinogen in some species and strains of rodents.

Risk modeling for carcinogenic xenobiotics has recently been segregated into three classes or types of models: physiologically based pharmacokinetic (PBPK) models in which the body is considered to be a small group of physiological compartments (Hoel et al., 1983; Krewski et al., 1986; Bischoff, 1987); biologically motivated models of carcinogenesis (BMMC) in which the carcinogenic process is considered to occur through a series of linked reactions that result from two or more molecular events followed by cellular amplification by "promoter" molecules (Moolgavkar, 1986; Thorslund et al., 1987; Krewski et al., 1987); and the linearized multistage model (LMS) of Armitage-Doll as modified by Crump and Howe (1984) in which it is assumed that a sequence of mutational events occur within a single cell leading to the neoplastic change (Armitage, 1985).

The model that appears to accommodate most of the critical components from the biological data base on 2,3,7,8-TCDD is the BMMC model, which is generally referred to as the Moolgavkar-Venzon-Knudson (M-V-K) model (Moolgavkar and Venzon, 1979; Moolgavkar and Knudson, 1981). This model allows for several of

the concepts of initiation-promotion-progression, along with the growth-stimulating role of endogenous substrates such as hormones (Moolgavkar, 1986). Incorporation of some of the factors necessary for the PBPK model can also be done using the M-V-K model as modified, or, more correctly, expanded by Thorslund et al. (1987). These expansions of the M-V-K model give the risk assessor a powerful tool for looking at cancer risk mechanistically.

This option is not available with the LMS model as originally proposed. The use of the LMS model may not be appropriate for the 2,3,7,8-TCDD data set since this model assumes an initiating event, such as a point mutation, to start the process. However, the LMS model can be accommodated if one hypothesizes that the initiating event: (1) is the result of an indirect action of 2,3,7,8-TCDD through modification of exogenous or endogenous compounds, (2) that a population of initiated cells exists, or (3) 2,3,7,8-TCDD leads to focal necrosis which serves as a mitogenic stimuli.

Recent reports have shown that 2,3,7,8-TCDD and other promoters in liver enhance stimulation of DNA synthesis in situ, and stimulate repair of O-6-methylguanine in liver DNA (Busser and Lutz, 1987; Den Engelse et al., 1986). Lutz et al. (1984) presented a scheme for promoter potency based on stimulation of DNA synthesis and the assumption that cell division is a prerequisite for several stages in the carcinogenesis process. These reports indicate that 2,3,7,8-TCDD can act as a complete-indirect-carcinogen, including promoter activity, despite the lack of DNA binding or direct mutagenesis. The sum of all these findings, along with the myriad of toxic responses, suggests a model for 2,3,7,8-TCDD carcinogenesis in rodent liver as shown in Figure 1. This model can account for the dose-response data in the bioassays and the multistage promotion experiments, as well as allow for incorporation into

existing risk models, and the scheme is not incongruent with the reports of decreased tumor formation in some tissues. If pathway (A) can be verified by demonstration of reactive intermediates after 2,3,7,8-TCDD treatment, then the LMS model, slightly modified, can be used. The preponderance of evidence at the moment supports a mechanistic model(s) which is at variance with the LMS model. However, Figure 1 presents possibilities that are not mutually exclusive for the existing models. The scheme also presents several testable hypotheses which should be examined.

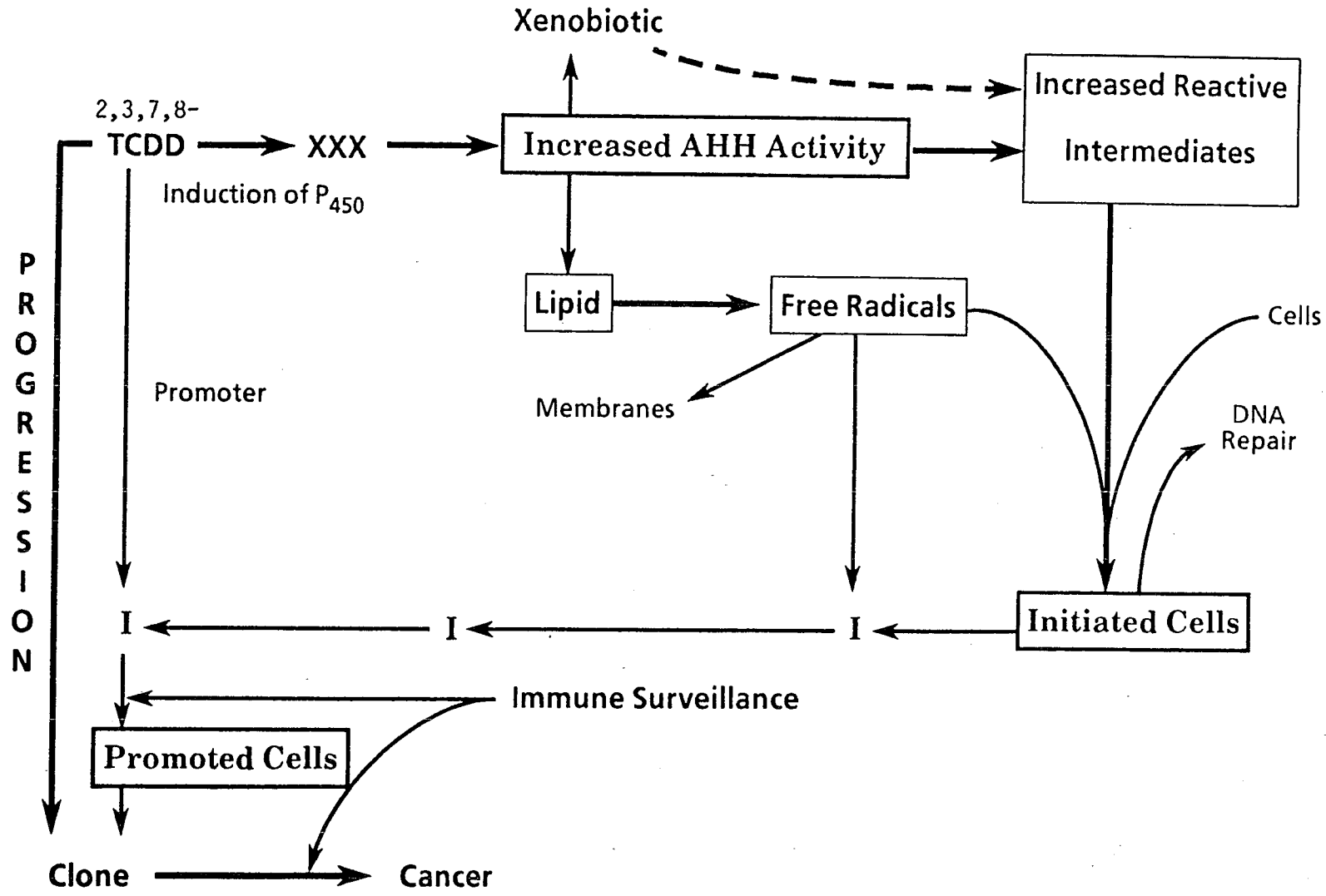


Figure 1. Potential secondary mechanisms of carcinogenic activity of 2,3,7,8-TCDD.

REFERENCES

Armitage, P. (1985) Environ. Health Perspect. 63:195-201.

Barsotti, D.A.; Abrahamson, L.J.; Allen, J.R. (1979) Bull. Environ. Contam. Toxicol. 21:463-469.

Becker, P.B.; Gloss, B.; Schmid, W.; Strahle, U.; Schultz, G. (1986) Nature (London) 324:686-688.

Berry, D.L.; DiGiovanni, J.; Juchau, M.R.; Bracken, W.M.; Gleason, G.L.; Slaga, T.J. (1978) Res. Commun. Chem. Pathol. Pharmacol. 20:101-107.

Bischoff, K.B. (1987) In: Drinking water and health 8:36-64. Washington, DC: National Academy Press.

Busser, M-T.; Lutz, W.K. (1987) Carcinogenesis 8:1433-1437.

Clark, A.J. (1933) The mode of action of drugs on cells. Baltimore, MD: Williams and Wilkins.

Crump, K.S.; Howe, R.B. (1984) Risk Analysis 4:163-176.

Den Engelse, L.; Floot, B.G.J.; Menkveld, G.J.; Tates, A.D. (1986) Carcinogenesis 7:1941-1947.

Denison, M.S.; Vella, L.M.; Okey, A.B. (1986) J. Biol. Chem. 261:3987-3995.

Dickson, R.B.; Lippman, M.E. (1987) Endocr. Rev. 8:29-43.

DiGiovanni, J.; Viaje, A.; Berry, D.L.; Slaga, T.J.; Juchau, M.R. (1977) Bull. Environ. Contam. Toxicol. 18:552-557.

Gallo, M.A.; Hesse, E.J.; Macdonald, G.J.; Umbreit, T.H. (1986) Toxicol. Lett. 32:123-132.

Gardner, R.M.; Kirkland, J.L.; Ireland, J.S.; Stancel, G.M. (1978) Endocrinology 103:1164-1172.

Gierthy, J.F.; Dickerman, H.W.; Seeger, J.L.; Lincoln, D.W.; Martirez, H.; Kumar, S.A. (1987) Toxicologist 7:159.

Goldstein, J.A.; Graham, M.J.; Sloop, T.; Maronpot, R.; Goodrow, T.; Lucier, G.W. (1987) Dioxin 87, Abstract # MA-07.

Gonzales, F.; Lakshman, S.; Hoath, S.; Fisher, D.A. (1984) Acta Endocr. 105:425-428.

Grossman, C.J. (1984) Endocr. Rev. 5:435-455.

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Official Business
Penalty for Private Use, $300

Please make all necessary changes on the above label, detach or copy, and return to the address in the upper left-hand corner.

If you do not wish to receive these reports CHECK HERE ☐; detach, or copy this cover, and return to the address in the upper left-hand corner.

EPA/600/6-88/007Ab