Comments of the Chlorine Chemistry Council on the National Toxicology Program's Listing of 2,3,7,8-TCDD in the Ninth Report on Carcinogens

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The Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, is pleased to submit these comments in response to the National Toxicology Program's request for public comments on substances proposed for listing or delisting in the 9th Report on Carcinogens (*63 Fed.Reg. 68783*). These comments provide an executive summary of CCC's previous comments regarding 2,3,7,8-TCDD (TCDD), and discuss relevant new developments.

CCC continues to believe that NTP should maintain its current classification of TCDD as a *reasonably anticipated* human carcinogen. There is insufficient epidemiological evidence of a causal relationship between TCDD exposure and cancer in humans. Such evidence is required by NTP's criteria for the *known* human carcinogen category. NTP's criteria do not permit NTP to overcome this insufficiency by relying on mechanistic data from animal studies to list TCDD as a *known* human carcinogen. The regulatory history of the 1996 revisions to the criteria demonstrates that NTP did not substantively change the criteria for the *known* human carcinogen category. The criteria, therefore, do not allow consideration of such mechanistic data to support a *known* listing.

Even if NTP were to consider mechanistic data, such data do not support reclassifying TCDD as a *known* human carcinogen. Apart from receptor binding and enzyme induction, little is known about mechanistic events leading to TCDD induced carcinogenicity in animals. Even less is known about TCDD's mechanism of action in humans. Therefore, the best available science does not meet NTP's criteria for classifying TCDD as a *known* human carcinogen.

NTP's Board of Scientific Counselor's Report on Carcinogens Subcommittee (ROC Subcommittee) reached this conclusion during its December 1998 review of TCDD. Their recommendation warrants great deference by NTP's Executive Committee.

NTP Must Maintain the Distinction between the *Known* and *Reasonably Anticipated* Human Carcinogen Categories.

NTP's criteria for the *known* category require sufficient evidence of carcinogenicity from studies in humans, which indicates a "causal relationship" between the agent and cancer. Concluding that a "causal relationship" exists requires that the available data meet important causation criteria. As discussed more fully in CCC's previous comments (summarized below), the available evidence falls significantly short of demonstrating a causal relationship between exposure to TCDD and cancer in humans.

Listing TCDD as a *known* human carcinogen would blur the important distinction between NTP's two categories in the Report on Carcinogens. The *reasonably anticipated* category is broad enough to accommodate substances with varying degrees of evidence for carcinogenicity. The *known* category is not. The *known* category would lose any significance if it included substances perceived as "likely" or even "almost known" to be carcinogens.

EPA's Science Advisory Board (SAB) recently highlighted the importance of maintaining a clear distinction between the *reasonably anticipated* and *known* human carcinogen categories. EPA's revised draft Cancer Risk Guidelines suggested that certain criteria short of sufficient human data (i.e., strong evidence of animal carcinogenicity, knowledge of the mode of carcinogenic action and associated key events in animals; and observation in exposed humans of the same key events preceding carcinogenicity

in animals) might support listing a substance a *known* carcinogen. During its January 1999 review of the draft Guidelines, the SAB determined that classifying substances as *known* carcinogens should be based solely on human data, either epidemiological data or human mode of action data.

The ROC Subcommittee's recommendation against reclassifying TCDD is entirely consistent with the SAB's views on EPA's Cancer Guidelines. Such harmonization of criteria among various federal agencies is necessary to create consistent chemical management policies. In a November 2, 1998 letter to Jim Tozzi of Multinational Business Services, Dr. Ken Olden stated "[EPA and HHS] are working together and with others to achieve harmonization; we agree that this is very important."

The Executive Committee Must Carefully Consider the ROC Subcommittee's Recommendation.

After reviewing the available data, the ROC Subcommittee voted 7-5 against listing TCDD as a *known* human carcinogen. The Subcommittee's public discussion and vote clearly demonstrate that no scientific consensus exists regarding TCDD's carcinogenicity in humans. This lack of consensus argues strongly for keeping TCDD listed as a *reasonably anticipated* human carcinogen.

The ROC Subcommittee's recommendation deserves great deference. The Subcommittee's vote occurred after the Subcommittee had reviewed TCDD twice, in October 1997 and again in December 1998. The Subcommittee members had more than a year to evaluate NTP's background materials on TCDD and cancer. The unprecedented second review also allowed the Subcommittee to assess the latest published studies, including two (Bertazzi and Hooiveld) that were discussed by Dr. Arnold Schecter at the October 1997 meeting, but were not yet available to the Subcommittee or the public.

Furthermore, the Subcommittee's review is the only portion of NTP's review process involving non-governmental scientists, and the only portion where the public is permitted to observe and contribute to the discussion. It provides the most robust, scientific review of NTP's recommendations for classifying substances as carcinogens. NTP adopted this external peer review so as to provide a formal scientific check on NTP's discretion in listing decisions. The fact that the ROC Subcommittee disagreed with the unanimous recommendations of RG1 and RG2 indicates a divergence between the federal government scientists and the broader scientific community on TCDD. This is precisely why outside peer review is sought.

The Executive Committee should follow the recommendation of the ROC Subcommittee unless there is a consensus within the broad, scientific community that runs counter to this recommendation. For TCDD, such a consensus does not exist. The disparate views among the ROC Subcommittee members are fairly representative of the scientific community at large. Indeed, EPA, after a lengthy and careful analysis, decided not to classify TCDD as a Group A carcinogen. The SAB supported this decision to consider TCDD to be, "probably carcinogenic to Humans with limited supporting information from human studies" (An SAB Report: A Second Look at Dioxin, p. 67). It would set a dangerous precedent for NTP to accept the recommendations of RG1 and RG2, and ignore the broader scientific community.

It is worth noting how NTP addressed saccharin after the ROC Subcommittee voted against the recommendations of RG1 and RG2. NIEHS Director Ken Olden felt compelled to solicit additional input from the scientific community before reaching a final decision that went against the recommendation of the subcommittee [See Dr. Olden's letter, **TAB 6**]. Dr. Olden's actions clearly illustrate the enormous scientific weight of Subcommittee's recommendations. In the case of TCDD, the second ROC Subcommittee review has provided ample time for input from outside scientists. At this time, any additional solicitation of comments is unlikely to demonstrate a scientific consensus on TCDD's carcinogenicity.

EXECUTIVE SUMMARY
OF COMMENTS PREVIOUSLY SUBMITTED TO NTP
BY THE CHLORINE CHEMISTRY COUNCIL

The Best Publicly Available and Peer-Reviewed Science Does Not Support Classifying TCDD as a *Known Human Carcinogen.*

NTP Criteria

- NTP's criteria for the *Known Human Carcinogen* category require sufficient evidence of carcinogenicity from studies in humans which indicates a "causal relationship" between the agent and cancer.

- NTP's criteria for the *known* human carcinogen category do not allow NTP to use data on a substance's "mechanism-of-action" to bolster limited evidence from studies in humans. When HHS revised the listing criteria in 1996, it did not substantively change the criteria for the "known" human carcinogen category. The notice (61 *Fed.Reg.* 50499 - September 26, 1996) shows that the only revisions made to the *known* category were the italicized editorial changes. All references to the use of mechanism of action data are under the section concerning revisions to the *reasonably anticipated* human carcinogen category. The Departmental press release and an *NIEHS News* article on the criteria confirm that substantive revisions to the criteria pertain only to the *reasonably anticipated* category.

- However, if mechanistic data is considered, the current mechanistic data do not support listing TCDD as a *known* carcinogen. Little is known about TCDD's mechanism of action in animals and even less is known about its mechanism of action in humans.

Epidemiological Evidence is Limited.

- Reviews by the Environmental Protection Agency (EPA), EPA's Science Advisory Board (SAB), and the International Agency for Research on Cancer (IARC) have concluded that available epidemiological data are "limited," and do not support classifying TCDD as a *Known Human Carcinogen*. [See **TAB 1**, CCC comments 8/25/97, p. 2]

- NTP's current list of *Known Human Carcinogens* includes only substances for which epidemiological data indicates a causal relationship between the substance and cancer. Establishing a causal relationship requires that data meet rigorous causation criteria, including strength of association, dose-response relationship, and consistency of findings. The weight of scientific evidence for TCDD does not adequately fulfill these well-recognized causation criteria. [See **TAB 1**, CCC comments 8/25/97, pp. 2-4]

Epidemiology Studies Reviewed in the Draft Dioxin Reassessment and by IARC Do Not Provide Sufficient Evidence that TCDD Causes Cancer in Humans.

- EPA cites four key epidemiological studies in its draft Dioxin Reassessment as the best evidence demonstrating the carcinogenicity of TCDD (Fingerhut et al. 1991, Saracci et al. 1991, Zober et al. 1990, and Manz et al. 1991). All report on workers occupationally exposed, and each suffers from confounding, inconsistency, and lack of specificity. For example, contrary to expectation, these studies of highly exposed occupational cohorts do not demonstrate carcinogenic effects more

conclusively than studies of less exposed cohorts. Further, even among these studies, there is a lack of consistency in the incidence of statistically significant cancer endpoints. [See **TAB 1**, CCC comments 8/25/97, pp. 4-6]

- The IARC Epidemiology Working Group considered the full body of epidemiological data on TCDD, including a number of studies published after the release of the draft Dioxin Reassessment. The group concluded that the epidemiological data remained "limited" and failed to demonstrate a causal relationship between exposure to TCDD and human cancer. [See **TAB 1**, CCC comments 8/25/97, pp. 7-9]

- The IARC Working Group noted three significant deficiencies in the epidemiological data. First, the statistical associations between exposure to TCCC and cancer risk are very weak. Second, the cancer outcome most consistently tied to TCDD exposure is the aggregation of all cancers combined, without specific cancers that predominate. Third, confounding by other exposures could not be excluded from some of the observed associations. [See **TAB 5**, comments of Dr. Raymond Greenberg on behalf of CCC 11/23/98, pp. 1-8].

- With this limited epidemiological data, IARC relied on mechanistic data to support its reclassification of TCDD as a "known" human carcinogen. Significant weaknesses with this mechanistic data are discussed below.

- Some epidemiological studies suggest that low to moderate TCDD exposure may not be carcinogenic to humans. In their 1995 study of the "Ranch Hand" cohort, Wolff et al. concluded

  > At the end of a decade of surveillance and more than 20 years after the last exposure to Agent Orange in Vietnam, Ranch Hands and Comparisons appear to be at equal risk for the development of all forms of neoplastic disease and there is no evidence to suggest a positive dose-response relationship between body burden of dioxin and neoplastic disease. [See **TAB 1**, CCC Comments 8/25/97, pp. 9-10]

Even Considering More Recent Studies, the Epidemiological Evidence Remains "Limited."

- The ROC Subcommittee considered two relevant epidemiology studies published after the October 1997 review (Hooiveld et al. (1998) and Flesch-Janys et al. (1998)). Because of significant weaknesses and methodological flaws, these studies provide no compelling data to change the overall assessment of the epidemiological data on TCDD as "limited." [See **TAB 3**, comments of Dr. Nathan Karch on behalf of CCC 6/15/98, pp. 5-9].

Current Information Concerning the Mechanism of Action for TCDD-Induced Carcinogenicity Does Not Support Listing TCDD as a *Known Human Carcinogen*.

- Presumably, if mechanistic data were used to move a substance from the *Reasonably Anticipated* to the *Known* human carcinogen list, NTP must demonstrate that the mechanism in animals is at least qualitatively similar in both animals and humans.

- Numerous statements from the draft Dioxin Reassessment and SAB's review demonstrate that TCDD's mechanism of action is not well understood in animals, and even less is known about mechanisms by which TCDD might induce cancers in humans. [See **TAB 1**, CCC Comments 8/25/97, pp. 10-19]

- TCDD binds to the Ah receptor and results in the induction of certain enzymes. Neither of these events is sufficient to explain how adverse responses, particularly cancer, occur. [See **TAB 1**, CCC comments 8/25/97, pp. 10-19]

- Beyond these two events, little is known about mechanistic events leading to carcinogenicity, even in the extensively studied rat liver tissue. In fact, there are no peer-reviewed publications that articulate the mechanism of action by which TCDD induces tumors in animals. [See **TAB 1**, CCC comments 8/25/97, pp. 10-19]

- Tumor production may involve mechanisms independent of Ah receptor binding. Several potentially Ah receptor-independent mechanisms are currently being investigated. [See **TAB 4**, comments of Dr. Nathan Karch on behalf of the Vinyl Institute 11/24/98, pp. 3-4]

- With only "limited" epidemiological data and poorly understood mechanism of action for TCDD-induced tumors in laboratory animals, NTP should continue to list TCDD as a *Reasonably Anticipated* human carcinogen.

Dioxin Levels In The Environment Have Fallen Dramatically Since Peaking In The Early 1970's

NTP's listing decisions are based on science, not policy considerations. It is worth noting, however, that dioxin levels in the environment have fallen dramatically since peaking in the early 1970's. TCDD and other dioxin congeners continue to be targets of emission standards and other regulatory actions. EPA's revised dioxin source inventory demonstrates that dioxin emissions to air and water fell more than 75% between 1987 and 1995. In addition, newly promulgated standards for municipal waste incinerators, medical waste incinerators, and pulp and paper mills, as well as proposed standards for hazardous waste incinerators and secondary aluminum smelters will provide substantial further emission reductions from the largest remaining sources.

Conclusion

Studies of various occupational populations exposed to TCDD have shown small elevations in total cancers, with underlying excesses in different tumors in different populations. There is little precedent in epidemiology for a causal risk factor to be associated with all types of cancer combined, without stronger associations to particular types of cancer. In addition, confounding cannot be excluded as an explanation for the small risk elevations detected in occupational cohorts. Exposure to other agents present in the occupational environment as well as smoking and other lifestyle habits may have resulted in the excesses. Given the presence of known carcinogens in most, if not all the occupational cohorts in which elevations have been observed, and given the lack of such generalized elevations in other populations with fewer confounding exposures (e.g., Seveso, Ranch Hand), the alternative exposures and causes must be systematically evaluated before the observed generalized excesses can be credibly attributed to TCDD exposure.

Unlike substances on NTP's current list of *known* human carcinogens, the epidemiological data for TCDD does not fulfill well-recognized causation criteria. Recent studies do not change the conclusions of IARC, EPA, and the SAB that the epidemiological data is "limited" or "suggestive." Even if NTP's criteria for the *known* category allow consideration of mechanistic data, TCDD's mechanism of action is too poorly understood to bolster the epidemiological data. NTP should continue listing TCDD as a *reasonably anticipated* human carcinogen.

Tab 1

CHLORINE
CHEMISTRY
COUNCIL

August 25, 1997

Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
MD WC-05, P.O. Box 12233
Research Triangle Park, NC 27709

VIA FACSIMILE AND FEDERAL EXPRESS

Dear Dr. Jameson:

A
Council
of the
Chemical
Manufacturers
Association

The Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, is pleased to submit the enclosed comments in response to NTP's evaluation of dioxin (2,3,7,8-TCDD) for the 9th Report on Carcinogens. CCC is dedicated to addressing public policy issues related to chlorine chemistry, and therefore has a significant interest in NTP's evaluation of TCDD.

As discussed more fully in the enclosed comments, the currently available epidemiology and mechanistic data are not sufficient for NTP to reclassify TCDD as a known human carcinogen. The epidemiological weight-of-evidence for TCDD does not adequately fulfill the well-recognized causation criteria. In fact, EPA, SAB, and IARC have independently concluded that the available epidemiological data on TCDD carcinogenicity are "limited" and do not support classification of TCDD as a known human carcinogen. NTP's revised listing criteria allow for consideration of mechanistic data. However, beyond Ah receptor binding and enzyme induction, the mechanism by which TCDD induces tumors in animals is not well understood. Even less is known about TCDD's mechanism of action in humans. Therefore, the available mechanistic data does not support classifying TCDD as a "known" human carcinogen.

Thank you for your consideration of these comments. If you have any questions regarding this submission, please contact Mr. David Fischer at (703) 741-5179.

Sincerely,

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Comments of the Chlorine Chemistry Council on the National Toxicology Program's Classification of 2,3,7,8-TCDD in the Ninth Report on Carcinogens

Introduction

The National Toxicology Program's (NTP's) current *Biennial Report on Carcinogens* (Report) lists 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as *Reasonably Anticipated to be a Humans Carcinogen.* For the ninth edition of this report, NTP is proposing to change the listing of TCDD to the *Known to Be a Human Carcinogen* category. In response to NTP's request for public comment, (62 Fed. Reg. 37,272 (July 11, 1997)), the Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, submits the following comments concerning the proposed TCDD listing.

NTP's recently revised criteria for listing chemicals as known human carcinogens (61 Fed. Reg. 50,499 (September 26, 1996)) requires "sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent . . . and human cancer." As discussed below, the epidemiology data for TCDD do not establish a causal relationship between TCDD exposure and cancer. The studies reporting an association between TCDD exposure and human cancers suffer from confounding, inconsistency, lack of specificity, and weak associations. Indeed, the U.S. Environmental Protection Agency (EPA), EPA's Science Advisory Board (SAB) and an International Agency for Research on Cancer (IARC) Epidemiology Working Group have concluded that the epidemiological data for TCDD are only "limited" or "suggestive." Therefore, TCDD epidemiology data do not support a change in NTP's current carcinogen listing for TCDD.

The recently revised NTP criteria also state that data related to a substance's mechanism of action may be considered. The criteria do not state whether mechanistic data could be used in place of epidemiological data that are not "sufficient" and, if so, how such data should be weighed. Before NTP can rely on mechanistic data for an animal carcinogen to assume carcinogenicity in humans, NTP must demonstrate that the substance exerts its carcinogenic effect through the same mechanism in both animals and humans. In the case of TCDD, sufficient mechanistic data do not exist.

CCC urges NTP not to change its current carcinogen listing for TCDD. NTP, however, should update its TCDD exposure and production discussion in its ninth edition of the Report. As discussed below, new TCDD production and exposure information demonstrates a dramatic decrease in TCDD production and exposure over the past decade, and TCDD production and emissions are anticipated to decrease further as new and proposed regulatory requirements are implemented.

The Epidemiological Data on TCDD-Induced Cancer in Human Populations Are Not Sufficient for NTP to Reclassify TCDD as a Known Human Carcinogen

The numerous epidemiological studies of human populations (even those exposed to unusually high levels of TCDD) do not demonstrate that TCDD is a known human carcinogen. Indeed, EPA, after a lengthy and careful analysis, decided not to classify TCDD as a Group A carcinogen. The SAB supported this decision, stating "...virtually all of the Committee believes that the studies of humans [would be categorized] as "limited" providing for an overall evaluation...as "Probably Carcinogenic to Humans with limited supporting information from human studies" (*An SAB Report: A Second Look at Dioxin,* p. 67). In addition, based on a thorough review of the epidemiology data, including a number of studies not available to EPA or the SAB during the preparation and review of the Draft *Dioxin Reassessment*, the IARC Epidemiology Working Group recently concluded that the epidemiological data were "limited" and therefore, by themselves, did not support a recommendation to designate TCDD a known human carcinogen. (IARC relied on mechanistic data to support its reclassification of TCDD as a "known" human carcinogen. Significant weaknesses with this mechanistic data are discussed below.)

The chemicals currently classified by NTP as known human carcinogens all have been placed in that category based on epidemiology data which satisfies the rigorous causation criteria established and recognized by the scientific community. There is no ongoing debate concerning whether any of these chemicals belongs on the list. The data are clear and unambiguous. The same standards should apply to TCDD or any other chemical which NTP considers classifying as a known human carcinogen.

NTP's category of *Known To Be A Human Carcinogen* is based primarily on sufficient

evidence of carcinogenicity from studies in humans which indicates a "causal relationship" between the agent and cancer. Causal relationships, by definition, imply that the data for any chemicals which are in this category meet the formal causation criteria, including strength of association, dose-response relationship, biological plausibility, consistency and specificity. Compounds currently classified by NTP as *Known To Be A Human Carcinogen* substantially fulfill the causation criteria. This group includes arsenic, asbestos, benzene, benzidine, bis(chloromethyl) ether, chloromethyl methyl ether, chromium (VI), coke oven emissions, diethylstilbestrol, lead arsenate, nickel, nickel sulfide roasting and dust, and vinyl chloride, Indeed, these substances are classified as known human carcinogens because the epidemiological data are of sufficient quality and quantity to fulfill these criteria. Table 1 illustrates the essential basis for including several representative chemicals in the known human carcinogen category.

Unlike NTP's current list of *Known Human Carcinogens*, the weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria. Indeed, EPA, SAB and IARC have concluded that the totality of the epidemiological data on TCDD carcinogenicity are only "limited" or "suggestive." Clearly, the data for the chemicals presently classified by NTP as *Known Human Carcinogens* rise above the level of being merely "limited" or "suggestive."

Table 1

Summary of Causation Criteria Fulfillment For Representative Chemicals Currently Classified by NTP as Known Human Carcinogens and TCDD

Compound	Consistent Findings	Strong Association	Dose-Response	Decreased Risk with Decreased Exposure
Arsenic and lead arsenate	+	+	+	+
Asbestos	+	+	+	-
Benzene	+	+	+	-
Benzidine	+	+	-	+
BCME & CMME	+	+	+	-
Chromium (VI)	+	+	+	-
Coke oven emissions	+	+	+	+ (?)
DES	+	+	-	-
Nickel, roasting and dust	+	+	+	+
Vinyl chloride	+	+	+	+
TCDD	-	-	-	-

Epidemiological Studies Reviewed in the Draft Dioxin Reassessment

EPA cites four key epidemiological studies in it's Draft *Dioxin Reassessment* as the best evidence demonstrating the carcinogenicity of TCDD (Fingerhut et al. 1991, Saracci et al. 1991, Zober et al. 1990, and Manz et al. 1991). All involve workers occupationally exposed, many of whom had chloracne (indicating very high exposures). The Fingerhut study reported an excess of respiratory cancers and all cancers combined. The authors concluded that the elevated risk for "all cancers combined" was consistent with the carcinogenic effects of TCDD observed in animal

studies. However, not a single tumor observed in the animal studies was found (i.e., cancers of the liver, biliary passages, gall bladder or nasal passages). Nor were cancers predicted from previous epidemiological studies (cancer of the stomach, Hodgkin's Disease, and non-Hodgkin's lymphoma) observed. Likewise, the studies by Saracci et al., Zober et al., and Manz et al. essentially failed to report "cancers of a priori interest." In reviewing these data, EPA concluded that "[t]he epidemiology evidence for a TCDD lung cancer hazard in humans is suggestive, but not conclusive, while that for all cancers combined has less certainty" (Draft Chapter 8, *Dose-Response Modeling,* p. 121).

The NIOSH Study By Fingergut et al.

Because of its size and detailed exposure data, the NIOSH study by Fingerhut et al. (1991) is generally held to be the best study of an occupational cohort highly exposed to TCDD. However, despite its size and detail, it is questionable whether the NIOSH study, because of the well recognized problems of confounding and bias, supports an association between TCDD exposure and cancer. Indeed, the SAB was skeptical about the results of this "best" study noting that workers "were exposed to a wide variety of potentially carcinogenic agents in addition to dioxin." The SAB stated that "[g]iven the possible confounding, and the somewhat equivocal links of dioxin to excess cancer in the group as a whole, it is difficult to document a dioxin-cancer relationship" (SAB, p. 51). Even the NIOSH study itself acknowledged that its findings could not "exclude a possible contribution from factors such as other occupational chemicals or smoking," that "other chemical exposures to which the workers were exposed may confound this analysis," and that "it is difficult to separate the effects of exposure to TCDD-contaminated products from the effects of exposure to numerous other chemicals encountered while employed at the plants" (Fingerhut et al. 1991, pp. 3, 18, and 28).

Exposures to other substances in the workplace could have easily accounted for any observed cancer increases in the NIOSH cohort. For example, smoking and exposure to asbestos could have accounted for the observed increase in lung cancers. Indeed, a misclassification of a single case of mesothelioma as a respiratory cancer would likely eliminate the statistical significance of the lung cancer finding attributed to TCDD exposure in the NIOSH high exposure cohort. Mesothelioma is typically under diagnosed in populations with no obvious exposure to

asbestos, so it is not unreasonable to hypothesize that one more case of mesothelioma might have escaped notice in the high exposure NIOSH cohort (Selikoff and Seidman 1992). This raises important questions concerning interactive effects between smoking and exposure to asbestos that could have substantially affected the interpretation that the moderately increased risk of lung cancer was due to TCDD exposure.

Other elements of the NIOSH study also suggest confounding by other chemicals. For example, the NIOSH study combined the results from 12 different manufacturing facilities in which workers could have been exposed to TCDD. However, all cancers and lung cancers were significantly elevated only at one plant (plant #10). The mortality experiences from Plant #10 contributed significantly to the overall pattern observed in the combined cohort. Removing the data from Plant #10 from the cohort brings the standard mortality ratio (SMR) for all cancers in the total cohort below statistical significance. Removal of data from Plant #10 also decreases the SMRs for all cancers and lung cancer in the high exposure cohort. Workers at this plant had a median 1.5 years in TCDD-contaminated processes and 21 additional years employed at the same plant with potential exposure to at least 13 additional chemicals classified by various agencies as potential human carcinogens. It is necessary to consider the contribution of other potentially carcinogenic chemicals to the incidence of cancer at plant #10. The NIOSH study, however, failed to do so.

Collectively, all of the studies of highly exposed populations reviewed by EPA in the Draft *Dioxin Reassessment* are more or less deficient in fulfilling three of the primary causation criteria (strength of association, consistent findings, and dose-response). For example, contrary to expectation, these studies of highly exposed occupational cohorts do not demonstrate effects more conclusively than studies of less exposed cohorts. Further, even among these studies, there is a lack of consistency in the incidence of statistically significant cancer endpoints. This is illustrated in Table 2.

Table 2

Lack of Consistency in Reported Associations Between Exposure to TCDD, and Cancer at Various Sites

Study	Cancer Type Reported				
	All Cancers	Lung	STS	Thyroid	Stomach
Fingerhut	+[6]	+[1,6]	+[5]	-	-
Saracci	-	-	-	+	+
Zober	-[3], +[4]	-	-	-	-
Manz	+[2]	+[1,2]	-	–	-

1 Confounded by smoking and exposure to asbestos
2 Not significant with both comparison control groups
3 Three cohorts based on job descriptions
4 Single cohort based on presence of chloracne or erythema
5 Cases only seen in 2 of 12 plants studied; significance questioned by authors
6 Only significant in 1 of 12 plants studied; 13 other potential carcinogens known to be present at same plant

Recent Epidemiology Studies Reviewed by IARC

A number of studies have been published since the release of the Draft *Dioxin Reassessment* and SAB's critical review of that document. The IARC Epidemiology Working Group considered many of these studies. In addition to the NIOSH study from the United States (Fingerhut et al. 1991), IARC also considered studies in the Netherlands (Bueno de Mesquita et al. 1993) and Germany (Ott and Zober 1996 and Becher et al. 1996), and an international study by IARC in which these and other data were aggregated (Kogevinas et al. 1996). In addition, IARC considered the most recent study of the Seveso cohort (Bertazzi et al. 1996). The IARC Epidemiology Working Group found that even these additional studies were not sufficient to alter the conclusion that the epidemiological data were still "limited" and failed to demonstrate a causal relationship between exposure to TCDD and human cancer. Each of these studies are briefly described below.

- Bueno de Mesquita et al. (1993) studied a cohort from the Netherlands. The cohort, part of the *IARC International Register of Persons Exposed to Phenoxy Herbicides and Contaminants,* was composed of 2310 workers engaged in the manufacture of phenoxy herbicides, chlorophenols and related compounds, many of which were contaminated with TCDD. Exposure estimates were based on detailed occupational history rather than on measurements of serum TCDD levels. No statistically significant increase in mortality due to all cancers combined or to respiratory cancer was observed. Significantly, the authors concluded that their findings "suggest that the increases in cancer mortality among workers exposed to phenoxy herbicides and chlorophenols may be attributable to chance" (Bueno de Mesquita et al. 1993, p. 289).

- Becher et al. (1996) studied four occupational cohorts from Germany consisting of a total of 2479 workers from four plants employed in manufacturing chlorophenoxy herbicides or chlorophenols. Exposure of the entire cohort was based on measured TCDD levels in a subsample of workers from two of the plants. The authors observed an increased mortality from all cancers combined in one of the four cohorts (SMR 134; CI 109-164). In addition, they reported an increased mortality from cancer of the buccal cavity and pharynx in one cohort (SMR 822; CI 300-1789), although this cohort was not the same one in which excess respiratory cancer was observed. Similarly, excess mortality from non-Hodgkin's lymphoma (NHL) was observed in two of four cohorts. Lung cancer (SMR 102; CI 102-213) or respiratory cancer (SMR 115; CI 115-230) was only elevated in one of the four cohorts. While smoking status was known for one cohort, this was not the cohort in which the elevated mortality from lung cancer was observed. Because these findings involve exposures to chemicals other than TCDD, it is difficult to attribute the observed effects to TCDD. For example, in discussing a slight excess of bladder cancer in one cohort, the authors noted that this could have been due to exposure to aromatic amines such as o-toluidine, ethyl toluidine, aniline, and anisidine which were known to have been used in that plant. Also, these findings are at odds with the reported findings of the NIOSH study in which no excess NHL or cancer of the buccal cavity and pharynx were reported, suggesting that different substances may have induced the cancers observed in the two cohorts.

- Ott and Zober (1996) reported cancer mortality in a small (N=243) occupationally exposed German cohort. Exposure was based on chloracne status and measured TCDD serum levels in a subsample of the cohort. Smoking status (never smoked, former smoker, current smoker) was based on a survey of an unknown number of cohort members. Although the authors reported 11 total cases of lung cancer, only one reported never having smoked cigarettes. The significance of the lung cancer findings is unclear since there was no TCDD dose-response trend among non-smokers. With respect to cancer mortality, the authors reported no statistically significant increases in any cancer associated with increasing exposure to TCDD. Finally, for cancer incidence, there were no statistically significant increased standardized incidence ratios (SIRs) for any cancer site associated with increasing TCDD dose levels. The authors of this study concluded, "[u]nfortunately, with such a small cohort, the risk estimates are not very stable and we are unable to assess whether TCDD might be exerting an influence independent of other

cancer risk factors." Thus, the results of this study provide little, if any, evidence that TCDD might be a human carcinogen. In addition, the authors' failure to find an increased incidence of all cancers combined or of respiratory cancer contradict the results of other studies.

- Bertazzi et al. (1996) provided an update of cancer mortality in the Seveso population exposed to TCDD after 15 years of latency. Exposure estimates were based on proximity to the accident with highest exposures in Zone A, intermediate exposures in Zone B and no exposure in Zone R. The authors reported no significant increase in respiratory cancer or in all cancer combined in men or women in any exposure zone. For women, they reported significant increased mortality from Hodgkin's disease and myeloma in Zone B, while for men they reported significant increased mortality from rectal cancer and leukemia in Zone B and increased mortality for esophageal cancer in Zone R. No increased cancer mortalities were observed in the highest exposed group. The authors of this study (which has not been peer reviewed) concluded only that "the observed departures from expectation, although based on small numbers of deaths, might be associated with dioxin exposure" (Bertazzi et al. 1996, p. 297).

- Kogevinas et al. (1996) reported cancer mortality in the IARC international retrospective cohort study of manufacturing workers or sprayers exposed to phenoxy herbicides. Reconstructed exposures were based on job descriptions or on TCDD serum measurements in some cohorts. For workers exposed to any phenoxy herbicide or chlorphenol, the authors reported a marginally significant increase in all cancers in men (SMR 1.07; CI 1.01-1.13), but not in women. In workers exposed to phenoxy herbicides contaminated with TCDD, the authors reported marginally significant increases in all cancer combined (SMR 1.6; CI 1.05-2.35) and breast cancer (SMR 2.22; CI 1.11-3.98). Due to the well recognized problems of confounding with exposures to other chemicals, the marginally significant results in this study (which has not been peer reviewed) are difficult to attribute to TCDD exposure.

The Ranch Hand Study

The Ranch Hand Study (Wolff et al. 1995), which IARC's Epidemiology Working Group did not consider, provides findings in a cohort moderately exposed to TCDD. The large cohort which comprises this study, while not as heavily exposed to TCDD as the occupational cohorts noted above, provides valuable information suggesting that cancer might not be an endpoint of concern for typical (even elevated) exposures to TCDD. In 1992, 20 years after the last exposure to TCDD, the Ranch Hand cohort demonstrated no statistically significant group differences for any neoplasm. Wolff et al. (1995) concluded that "at the end of a decade of surveillance and more than 20 years after the last exposure to Agent Orange in Vietnam, Ranch Hands and Comparisons appear to be at equal risk for the development of all forms of neoplastic

disease and there is no evidence to suggest a positive dose-response relationship between body burden of dioxin and neoplastic disease."

Conclusion

The body of epidemiology evidence for TCDD, including recent studies examined by IARC, do not provide sufficient evidence that TCDD causes cancer in humans. Some human epidemiology studies, such as the Ranch Hand study (Wolff et al., 1995), suggest that low to moderate TCDD exposure may not be carcinogenic to humans. The evidence concerning the potential carcinogenic effects associated with high human TCDD exposures, on the other hand, is not clear. Studies of highly exposed industrial cohorts typically suffer from confounding by exposures to a wide variety of industrial chemicals (many of which are known to be potential carcinogens), inconsistency, and lack of specificity. The finding of "new" cancers in different cohorts, the failure to confirm key findings from previous studies (e.g., lung cancer), and the finding of only marginally elevated SMRs in cohorts exposed to TCDD levels do not provide a sufficient basis from which to conclude that TCDD is a known human carcinogen.

Current Information Concerning the Mechanism of Action for TCDD-Induced Carcinogenicity Does Not Support Changing the TCDD Carcinogenicity Listing

NTP's recently revised listing criteria state that mechanism of action may also be considered when drawing conclusions regarding carcinogenicity in humans or experimental animals. It is not clear whether mechanistic data could be used when epidemiological data are insufficient to move a chemical from the *Reasonably Anticipated to be a Human Carcinogen* to the *Known Human Carcinogen* list. Indeed, the only example provided in the revised criteria concerns removing an animal carcinogen from listing when evidence demonstrates that the substance, for mechanistic reasons, is not anticipated to be carcinogenic in humans.[1]

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[1] To date, very few chemicals have "cleared the hurdle" with respect to being classified (or not classified) as anticipated tobe human carcinogens or known human carcinogens based on mechanism of action data. Perhaps the best example of how data on mechanism of action have been used to **not** classify certain chemicals as potential human carcinogens concerns the issue of $\alpha_{2\mu}$-globulin nephropathy. For a certain class of chemicals (e.g., unleaded gasoline), chronic exposure results in nephropathy and renal tumors in male rats and no effects in female rats or in either sex of any other species. The nephropathy and tumor formation results from the

Presumably, if mechanistic data were used to move a substance from the *Reasonably Anticipated to be a Human Carcinogen* to the *Known Human* Carcinogen list, NTP must demonstrate that the mechanism in animals is at least qualitatively similar in both animals and humans. This would require a detailed understanding of the mechanism of action for a specific substance in both the experimental animals and humans. If NTP relied instead on inference and assumptions, it would likely find that most of the *Anticipated to be Human Carcinogens* would be upgraded to *Known Human Carcinogens*, thereby losing the important (and statutorily required) distinction between the two categories.

In the case of TCDD, little is known about its mechanism of action in animals and even less is known about its mechanism of action in humans. Therefore, current mechanistic data cannot support moving TCDD to the *Known Human Carcinogen* list. As discussed below, the current hypotheses concerning possible TCDD tumor-inducing mechanisms are merely speculation. What is known and generally accepted at this time concerning TCDD mechanisms of actions is that TCDD, like a number of other substances, most of which are not "known to be carcinogenic to humans," binds to the aryl hydrocarbon (Ah) receptor and results in the induction of certain enzymes. Neither of these events, however, are sufficient to explain how adverse responses, particularly cancer, occur. Apart from receptor binding and enzyme induction, little, if anything, is known, much less understood, about mechanistic events leading to TCDD-induced

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accumulation of $\alpha_{2\mu}$-globulin, a protein specific to male rats only. Since humans of both sexes lack this protein, there is no possibility of $\alpha_{2\mu}$-globulin-mediated renal nephropathy occurring in humans. Based on this well documented and unequivocal understanding of the mechanism of action, EPA's regulatory policy for chemicals that only induce renal tumors in male rats by this mechanism is that they do not pose a carcinogenic risk to humans. NTP's revised descriptive criteria for determining the potential carcinogenicity of chemicals implicitly endorses this body of data ("*...there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore reasonably be anticipated not to cause cancer in humans.*) This detailed and unequivocal understanding of the mechanism of action by which certain chemicals induce tumors in animals (and the validation of this in numerous peer reviewed publications) is in contrast to the current understanding of the mechanism of action by which TCDD might cause tumors in animals, much less in humans.

carcinogenicity in animals. Even less is known about the mechanisms by which TCDD might induce toxicity in humans.

The Draft Dioxin Reassessment Demonstrates That Little is Known About the Mechanism of Action by which TCDD Causes Cancer

EPA, after extensively reassessing TCDD, has acknowledged that little is known about the mechanism by which TCDD exerts toxicity (including cancer) in animals. For example, in it's Draft *Dioxin Reassessment* EPA states:

> [i]t has repeatedly been reported as the current opinion that all known effects of TCDD are probably Ah receptor mediated. However, except for the chain of events leading to the induction of certain enzymes, clear evidence for such a conclusion is still lacking" (p. 3-36).

A true mechanism of action for a chemical must be both necessary and sufficient with respect to explaining how the chemical induces toxicity in animals. For the two events of Ah receptor binding and enzyme induction for TCDD, this is clearly not the case. EPA has stated that "[b]inding to the Ah receptor appears to be necessary for all well-studied effects of dioxin but is not sufficient, in and of itself, to elicit these responses" (Draft *Dioxin Reassessment,* p. 9-78). It is clear from this statement that the mechanism of action by which TCDD induces toxicity is unknown. If binding to the Ah receptor is not sufficient, then some other explanation must address the critical data gap between Ah receptor binding and toxicity induction. Presently, there is little if any knowledge of events past Ah receptor binding which are involved with TCDD-induced carcinogenicity.

A number of statements by EPA (in it's Draft *Dioxin Reassessment* and in recent revisions of that document) and by the SAB (in reviewing the Draft *Dioxin Reassessment*) support the conclusion that Ah receptor binding and enzyme induction do not, in and of themselves, explain how adverse responses, particularly cancer, occur in animals. These statements also demonstrate that little is known about the mechanistic events leading to TCDD-induced carcinogenicity in animals and even less is known about the mechanism by which TCDD might induce toxicity in humans.

EPA states in the Draft *Dioxin Reassessment*:

In certain cases, no response occurs even when there is some receptor occupancy, a

> threshold phenomenon that reflects the biological "inertia" of the system (Ariens et al. 1960). In other cases a maximal response occurs well before all receptors are occupied, a phenomenon which reflects receptor "reserve" (Stephenson, 1956). Therefore, one cannot simply assume that the relationship between fractional receptor occupancy and biological response is linear. Furthermore, for a ligand (such as TCDD) that elicits multiple receptor-mediated effects, one cannot assume that the binding response relationship for one, simple effect (such as enzyme induction) will necessarily be identical to the binding response relationship for a different effect (such as cancer). (Chap. 2, at 2-5).

This statement undermines any conclusion that Ah receptor binding somehow "explains" the carcinogenicity of TCDD. If the binding response relationships for enzyme induction and cancer are different, it cannot be concluded with any confidence that the mechanism of action by which TCDD-induced carcinogenicity occurs is adequately understood.

Even assuming that the mechanism of carcinogenic action of TCDD in animals involves the Ah receptor, the lack of knowledge concerning the similarities and differences between human and animal Ah receptors undermines any speculation that the mechanism for the carcinogenicity of TCDD in animals applies to humans. According to EPA, there is great uncertainty on this critical point. EPA states that "[t]he human receptor has not been studied extensively, and it is unknown if the properties of the human protein differ substantially from those of the Ah receptor in animals (Draft *Dioxin Reassessment*, p. 2-8).

Further, EPA has concluded that "there is no clear mechanistic link between CYP1A1 induction and cancer" (Draft *Dioxin Reassessment,* p. 6-27). Because many chemicals, in addition to TCDD, can induce enzymes (e.g., CYP1A1), the role of this event in explaining carcinogenicity is unknown (particularly for non-genotoxic chemicals like TCDD). The fact that there is no clear link between Ah receptor-mediated CYP1A1 induction and cancer suggests that the mechanism by which TCDD induces cancer in animals is unknown.

The above examples highlight the fundamental uncertainty expressed in the Draft *Dioxin Reassessment* concerning the lack of understanding concerning the mechanism of TCDD-induced carcinogenicity. It is clear that beyond Ah receptor binding, the mechanism of action for TCDD-induced tumors in animals is poorly understood.

SAB's Review of the Draft Dioxin Reassessment Demonstrates Little is Known About the Mechanism of Action by Which TCDD Causes Cancer

The SAB's review of the Draft *Dioxin Reassessment* also supports the conclusion that the mechanism of TCDD-induced carcinogenicity is unknown or, at best, is poorly understood. The following excerpts from the SAB review clearly illustrate this point:

- "Chapter 2 [of the Draft *Dioxin Reassessment*] offers an unequivocal assessment that the Ah receptor mediates the biological effects of TCDD. Yet, when reading Chapters 3, 4 and 5 dealing with specific toxic events, it becomes clear that numerous fundamental uncertainties occur and mechanisms of action for the toxic events beyond receptor binding are largely unknown" (p. 49).

- "Much of what is purported to link the Ah receptor to specific toxic events is merely the demonstration of an association between the binding of TCDD to the receptor and an eventual appearance of an adverse effect some time later in some species.... But the possible downstream events, if they exist, between Ah receptor binding and the final toxic manifestation are not well established. Mechanism of action should mean that at least some of the intermediate steps, after Ah receptor binding and leading to the pathologic processes involved, are known to some extent.... In actual fact, the only mechanism of action involving the Ah receptor that has been worked out sufficiently well to be called the biological sequence that describes a "mechanism of action" is the induction of cytochrome P450.....The rest of the biological consequences of TCDD exposure are yet to be described adequately and sequentially in mechanistic terms" (p. 49).

- "[T]here is a large intellectual chasm between the elegant science describing the details of the TCDD receptor and its mechanism of initiating a cellular response, and the poorly understood manifestations of the toxic events associated with an alteration of the homeostasis of an animal. The linkage between Ah receptor action and specific cellular toxicity remains undefined.... In any future revisions, EPA should present more clearly the major deficiencies that exist in the current mechanism database and provide some discussion of any plausible alternative hypotheses" (p. 50).

- "Most of the "mechanistic data" support the involvement of the Ah receptor, but say little (in the context of toxicity), about how the activation of this protein alters normal physiologic function and/or development. Risk assessments based solely on Ah receptor activation or on the existing knowledge of CYP1A1 induction are unlikely to provide a biologically defensible prediction (quantitatively or qualitatively) of likely toxic outcomes in humans, particularly under low exposure scenarios" (p. 49).

- Concerning EPA's conclusion about the role that the purported mechanisms of action might contribute to the diversity of biological response seen in animals and, to some extent, in humans "can be better posed as...how convincing is the evidence for the purported mechanisms that link receptor binding to toxic effects in humans? Unfortunately, the

evidence is quite mixed" (p. 51).

- "The statement [EPA's conclusion that there is a continuum of responses, based, in part on speculations about mechanism of action] is only defensible in reference to a limited number of specific case examples, but cannot be taken as universally proven. Until a full mechanism of action has been mapped out, the reassessment's position remains unproved in general. The statement should not be presented as a "postulate (which is widely accepted as a universal truth not requiring proof) but as a current hypothesis (subject to change as new data are discovered)" (p. 79).

From the numerous above statements taken from the SAB's review of the Draft *Dioxin Reassessment* there can be no doubt that the mechanism of action by which TCDD induces tumors in animals is neither known nor understood. Given this uncertainty, it would be premature for NTP to proceed with the proposed reclassification of TCDD to a known human carcinogen based on mechanism of action considerations.

EPA's Recent Revisions to the Draft Dioxin Reassessment

EPA's recent revised Chapter 8 of the *Dioxin Reassessment* (Dose-Response Modeling, January 27, 1997) and it's partially revised risk characterization for the *Dioxin Reassessment* (Draft *Integrated Risk Characterization for TCDD,* Sept. 24, 1996) continue to demonstrate that the mechanism of action by which TCDD might induce tumors in laboratory animals is still unknown.

While Chapter 8 recognizes that TCDD binds to the Ah receptor, EPA expresses considerable uncertainty concerning this event. For example, EPA acknowledges that "[t]he relationship between Ah receptor binding and carcinogenicity of TCDD is less clear" (Revised Chap. 8, p. 6). EPA also states that, "[t]he induction of CYP1A proteins are perhaps the best characterized responses to dioxins. The relevance of these proteins to the toxic effects of TCDD are controversial" (Revised Chap. 8, p. 91). EPA also states:

- "Most of the mechanistic or dose-response information on dioxin's effects has been generated on changes in gene expression of single genes such as CYP1A1 induction. There is only limited information on the complete interaction of biochemical, molecular, and biological events that are necessary to produce a frank toxic effect such as cancer..." (p. 129).

- "The development and implementation of a complete mechanistic [risk assessment] model for the effects of TCDD identified several areas where future research is needed. Of critical utility would be data and models which are able to directly link gene expression with toxicity

in a mechanistic fashion" (p. 141).

In it's Draft *Integrated Risk Characterization for TCDD,* EPA further illustrates how poorly the mechanism of action by which TCDD might induce tumors in laboratory animals is understood. EPA states:

- "The fact that TCDD **may induce** a cascade of biochemical changes in the intact animal **raises the possibility** that dioxin **might produce** a response such as cancer by mechanisms that differ among tissues....One **possible mechanism** discussed is that TCDD **might activate** a gene(s) that is directly involved in tissue proliferation. A second mechanism involves TCDD-induced changes in hormone metabolism, which **may lead** to tissue proliferation ...which **might lead** to indirect mutagenic effects. Thus, while this reassessment as identified a number of **hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity, and in which species**" (p. 72, emphasis added).

- "The ability of TCDD...to modulate a number of biochemical parameters in a species-, tissue-, and temporal specific manner is well recognized. Despite the ever expanding list of these responses over the past 20 years and the elegant work on the molecular mechanisms mediating some of these, there still exists a considerable gap between our knowledge of these changes and the degree to which they are related to the biological and toxic endpoints elicited by these chemicals" (pp. 74-5).

- "Thus, while this reassessment has identified a number of hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity and in which species" (p. 72).

- "Thus, the mechanisms by which many, if not most, of the biochemical processes are altered by TCDD treatment remain to be determined. Nevertheless, it is presumed based on the cumulative evidence available...that all of these processes are mediated by the binding of TCDD to the Ah receptor" (p. 75).

Based upon these statements, it is simply not possible to support an argument that the mechanism by which TCDD induces cancer in laboratory animals (much less in humans) is known or understood.

Speculation Concerning Possible Mechanisms

The lack of knowledge concerning the mechanism of action by which TCDD induces tumors in animals, however, has not prevented EPA and others from speculating on a possible mechanism. For example, in the Draft *Dioxin Reassessment,* EPA speculates that the

carcinogenic actions of TCDD may involve hormones. EPA states that "the carcinogenic actions of TCDD involve a complex interaction of hormonal factors" (p. 9-43). However, a description of the sequence of hormonal events occurring after Ah receptor binding which lead to the carcinogenicity of TCDD in animals has never been published. Nor has the possible relevance of these events in humans. Even if hormone interactions play some role in high dose animal carcinogenicity, the role of hormone interactions, in potential human carcinogenicity is, at best, speculative. (Indeed, there is evidence that certain hormonal effects of TCDD act as an anti-carcinogen in humans at low doses.) The unknown nature of this aspect of TCDD carcinogenesis only adds to the uncertainty concerning the potential mechanism of action for TCDD.

EPA also speculates in it's Draft *Dioxin Reassessment* that there may exist some interplay between enzyme induction, hormonal effects and subsequent carcinogenicity. EPA states that:

> ...**it has been hypothesized** that increases in UDP-glucuronyltransferases leads to elimination of thyroxine and **may lead indirectly** to increased TSH synthesis...and hypertrophic responses by the thyroid. **There is speculation** that such prolonged stimulation **may lead** to the thyroid tumors seen in both rats and mice exposed to TCDD. **Data to confirm this effect of dioxin...in humans are not available** (p. 9-52, emphasis added).

The above speculation concerning a possible mechanism of action is not borne out by data. Thyroid cancer has almost never been reported in the TCDD-exposed cohorts studied. For example, in the NIOSH study (Fingerhut et al. 1989) there were no thyroid cancer deaths. Thus, empirical data demonstrates that this speculative mechanism of action for TCDD-induced tumors may be incorrect.

NTP Should Publish A Detailed Basis For Any Decision In Which Mechanism Of Action Considerations For TCDD-Induced Carcinogenesis Are Employed

Although IARC has classified TCDD as a known human carcinogen based on mechanistic considerations, a detailed explanation for this decision has yet to be published and subject to review by the scientific community. Therefore, it is not clear how IARC's decision can comport with the lack of scientific evidence. A decision by NTP to rely on mechanism of action data to raise TCDD to the category of *Known Human Carcinogen* must be based on a scientific explanation acceptable to the scientific community. Given the considerable uncertainties (and

controversy) surrounding this issue, and the general lack of any peer reviewed and published papers on the subject, it is incumbent upon NTP to publish a detailed basis for any decision in which mechanism of action considerations for TCDD-induced carcinogenesis are employed.

Conclusion

Based upon numerous statements from the Draft *Dioxin Reassessment*, the SAB's comments on the Draft *Dioxin Reassessment,* and on EPA's revised versions of Chapter 8 on Dose-Response Modeling as well as the Draft *Integrated Risk Characterization for TCDD*, it is clear that the mechanism of action by which TCDD induces tumors in laboratory animals is unknown or, at best, uncertain. While it is universally accepted that TCDD binds to the Ah receptor, beyond this initial event, the sequence of events leading to tumor development is essentially unknown. In fact, there are no peer reviewed publications which articulate the mechanism of action by which TCDD induces tumors in animals.

TCDD Production and Environmental Concentrations Have Decreased Significantly Over the Last Decade

TCDD has never been intentionally produced other than on a laboratory-scale basis for use in toxicity studies. Rather, it is generated as a by-product of various combustion (some naturally occurring) and chemical processes. Over the last decade TCDD production and environmental concentrations have dramatically decreased due in large part to the discontinued production and use of the herbicide 2,4,5-T, reductions in the manufacture of other chlorinated phenolic compounds, the switch to unleaded automobile fuels, upgraded emission controls for incinerators, and process changes at pulp and paper mills. Additional significant decreases in TCDD production and emissions will result from implementation of recently promulgated emission standards for municipal and medical waste incinerators, and from proposed emission standards for hazardous waste incinerators and for the pulp and paper industry.

EPA has recently conducted a dioxin inventory that assessed dioxin releases to the environment from 1987 to 1995. Although that data has not been published (EPA anticipates releasing this information before the end of the year), it is our understanding that EPA will demonstrate a greater than 70% decrease in dioxin releases for that time period. EPA's estimate

does not include decreased releases since 1995 and the very significant projected emission decreases that will result from compliance with new and proposed regulatory standards. NTP should update its exposure discussion for TCDD to include this new information. NTP may wish to discuss the dioxin inventory data and the projected impact of the new and proposed emission standards with EPA's Office of Research and Development and with relevant EPA Program Offices.

Conclusion

Unlike substances on NTP's current list of *Known Human Carcinogens*, the epidemiological weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria. Despite a great deal of study on numerous populations exposed to varying amounts of TCDD (some of them having extremely high exposure), the epidemiological data do not permit a conclusion that TCDD is a known human carcinogen. Indeed, three independent reviews (EPA, SAB and IARC) have concluded that the totality of the epidemiological data on TCDD carcinogenicity are only "limited" or "suggestive." Such data do not provide "sufficient evidence" to allow NTP to classify TCDD as a *Known Human Carcinogen.*

The poorly understood mechanism of action for TCDD-induced tumors in laboratory animals, in conjunction with "limited" or "suggestive" epidemiological data, do not support listing TCDD as a known human carcinogen. EPA's Draft *Dioxin Reassessment*, the SAB's extensive review of that document, as well as EPA's revisions to the Draft *Dioxin Reassessment* are all in agreement that beyond Ah receptor binding and enzyme induction, the mechanism by which TCDD induces tumors in animals is poorly understood. At present, there is a significant data gap between Ah receptor binding and enzyme induction and the unknown events which are necessary for the development of tumors. Based on the totality of the available data (epidemiology and mechanism of action), the most that can be concluded is that TCDD can be reasonably anticipated to be a human carcinogen. Clearly, the available data do not support NTP's proposed changing of its current listing of TCDD to the "Known to Be a Human Carcinogen" category.

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Tab 2

CCC's Comments to NTP's
Board of Scientific Counselors
Report on Carcinogens Subcommittee

Submitted Via Facsimile
October 27, 1997

Comments of the Chlorine Chemistry Council on the National Toxicology Program's Classification of 2,3,7,8-TCDD in the Ninth Report on Carcinogens

Introduction

The National Toxicology Program's (NTP's) current *Biennial Report on Carcinogens* (The Report) lists 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as *Reasonably Anticipated to be a Human Carcinogen*. For the ninth edition of this report, NTP is proposing to change the listing of TCDD to the *Known to be a Human Carcinogen* category. The Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, believes that the science does not support this proposed change, and urges the NTP Board of Scientific Counselors to advise NTP not to list TCDD as a known human carcinogen. CCC submits the following brief comments concerning the proposed TCDD listing. CCC submitted more extensive comments to NTP in August 1997 (CCC's August Comments).

NTP's recently revised criteria for listing chemicals as known human carcinogens requires "sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent . . . and human cancer" (61 Fed. Reg. 50,499 (September 26, 1996)). As demonstrated below and discussed more fully in CCC's August Comments, the epidemiology data for TCDD do not establish a causal relationship between TCDD exposure and cancer. The studies reporting an association between TCDD exposure and human cancers suffer from confounding, inconsistency, lack of specificity, and weak associations. Indeed, the U.S. Environmental Protection Agency (EPA), EPA's Science Advisory Board (SAB) and an International Agency for Research on Cancer (IARC) Epidemiology Working Group have concluded that the epidemiological data for TCDD are only "limited" or "suggestive." Therefore, TCDD epidemiology data do not support a change in NTP's current carcinogen listing for TCDD.

The recently revised NTP criteria also state that data related to a substance's mechanism of action may be considered. Beyond binding with the Ah receptor, the mechanism of carcinogenic

action for TCDD is unknown. Therefore, there is no mechanistic basis for assuming that TCDD is carcinogenic in humans simply because it has been found to be carcinogenic in some animal studies. In fact, the dramatic differences between the half lives of TCDD in animals and humans (e.g., 10 days versus 10 years) may demonstrate a significant biochemical difference between animals and humans.

NTP's criteria are clear: when there is evidence of carcinogenicity in animals, limited evidence in humans, and insufficient mechanistic data, a substance is to be classified as *Reasonably Anticipated to be a Human Carcinogen*.

The Epidemiological Data for TCDD Are Not Sufficient to Support Listing TCDD as a Known Human Carcinogen

Unlike NTP's current list of known human carcinogens, the weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria (e.g., consistent findings, a strong association, and evidence of a dose-response relationship). Indeed, EPA, the SAB and IARC have concluded that the totality of the epidemiological data on TCDD carcinogenicity are only "limited" or "suggestive."

The numerous epidemiological studies of human populations (even those exposed to unusually high levels of TCDD) do not demonstrate that TCDD is a known human carcinogen. EPA, after a lengthy and careful analysis (which included extensive input from the scientific community), decided not to classify TCDD as a Group A carcinogen. The SAB supported this decision, stating "...virtually all of the Committee believes that the studies of humans [would be categorized] as 'limited' providing for an overall evaluation...as 'Probably Carcinogenic to Humans' with limited supporting information from human studies" (*An SAB Report: A Second Look at Dioxin,* p. 67).

EPA cites four key epidemiological studies in it's Draft *Dioxin Reassessment* as the best evidence demonstrating the carcinogenicity of TCDD (Fingerhut et al. 1991, Saracci et al. 1991, Zober et al. 1990, and Manz et al. 1991). All involve workers occupationally exposed, many of whom had chloracne (indicating very high exposures). The Fingerhut study reported an excess of respiratory cancers and all cancers combined. The authors concluded that the elevated risk for

"all cancers combined" was consistent with the carcinogenic effects of TCDD observed in animal studies. However, not a single tumor observed in the animal studies was found, nor were cancers predicted from previous epidemiological studies observed. Likewise, the studies by Saracci et al., Zober et al., and Manz et al. essentially failed to report "cancers of a priori interest." In reviewing these data, EPA concluded that "[t]he epidemiology evidence for a TCDD lung cancer hazard in humans is suggestive, but not conclusive, while that for all cancers combined has less certainty" (*EPA Draft Dioxin Reassessment Revised Chapter 8, Dose-Response Modeling,* p. 121). These studies, including EPA's and the SAB's findings concerning these studies, are discussed in more detail in CCC's August Comments.

The RC Background Document for TCDD (RC Document) claims, "the strongest epidemiological evidence for the carcinogenicity of 2,3,7,8-TCDD is for all cancers combined, rather than for any specific site"(Appendix A, p. 337). Yet even for this finding, the relative risk of 1.4 does not constitute compelling evidence, particularly when confounding cannot be excluded. Furthermore, known human carcinogens (e.g., smoking, ionizing radiation) which produce tumors at more than one site also demonstrate unequivocal elevated risks for specific sites. In fact, there is no known compound that is a multi-site carcinogen without particular sites predominating (Id.). This strongly suggests that the epidemiological evidence for TCDD is more consistent with exposures to multiple chemicals rather than exposure to a single chemical, such as TCDD.

A number of studies have been published since the release of the Draft *Dioxin Reassessment* and SAB's critical review of that document. An IARC Epidemiology Working Group conducted a thorough review of the epidemiological data, including the NIOSH study from the United States (Fingerhut et al. 1991), studies from the Netherlands (Bueno de Mesquita et al. 1993) and Germany (Ott and Zober 1996 and Becher et al. 1996), an international study by IARC in which these and other data were aggregated (Kogevinas et al. 1996), and the most recent study of the Seveso cohort (Bertazzi et al. 1996). The Working Group found that even these additional studies were not sufficient to alter the conclusion that the epidemiological data were "limited" and failed to demonstrate a causal relationship between exposure to TCDD and human cancer. These studies, and their limitations are also discussed in CCC's August Comments.

Current Information Concerning the Mechanism of Action for TCDD-Induced Carcinogenicity Does Not Support Changing the TCDD Carcinogenicity Listing

NTP's recently revised listing criteria state that mechanism of action may also be considered when drawing conclusions regarding carcinogenicity in humans or experimental animals.[1] Presumably, if mechanistic data were used to move a substance from the *Reasonably Anticipated to be a Human Carcinogen* list to the *Known to be a Human Carcinogen* list, NTP must demonstrate that the mechanism in animals is at least qualitatively similar in both animals and humans. This would require a detailed understanding of the mechanism of action for a specific substance in both experimental animals and humans.

In the case of TCDD, little is known about its mechanism of action in animals, and even less is known about its mechanism of action in humans. Therefore, current mechanistic data cannot support moving TCDD to the *Known to be a Human Carcinogen* list. As discussed below, the current hypotheses concerning possible TCDD tumor-inducing mechanisms are merely speculation. What is known and generally accepted at this time concerning TCDD mechanisms of actions is that TCDD, like a number of other substances, binds to the aryl hydrocarbon (Ah) receptor and results in the induction of certain enzymes. Neither of these events, however, are sufficient to explain how adverse responses, particularly cancer, occur. Apart from receptor binding and enzyme induction, little is known, much less understood, about mechanistic events leading to TCDD-induced carcinogenicity in animals. Nonetheless, the RC Background Document for TCDD (RC Document) relies on Ah receptor binding to incorrectly imply that animals and humans share the same mechanism of carcinogenic action for TCDD.

Not only is there insufficient information to conclude that animals and humans share the

[1] It is not clear whether mechanistic data can be used when epidemiological data are insufficient to change the listing of a chemical from *Reasonably Anticipated to be a Human Carcinogen* to *Known to be a Human Carcinogen*. Indeed, the only example provided in the revised criteria concerns removing an animal carcinogen from listing when evidence demonstrates that the substance, for mechanistic reasons, is not anticipated to be carcinogenic in humans.

same mechanism of carcinogenic action for TCDD, some evidence suggests that animals and humans may exhibit important mechanistic differences after initial Ah binding. As discussed elsewhere in the RC Document, TCDD has a strikingly different half-life in rodents than in humans (10-30 days compared 5.8-11.3 years, respectively). This important distinction suggests that animals and humans do not share the same mechanism of carcinogenic action for TCDD because TCDD is metabolized differently, possibly through different enzyme systems or transcriptional activity. Further, if TCDD exerts a carcinogenic effect in animals through the activity of a metabolite, rodents may be much more sensitive to TCDD than humans.

A true mechanism of action for a chemical must be both necessary and sufficient with respect to explaining how the chemical induces toxicity in animals. This is clearly not the case for Ah receptor binding and enzyme induction for TCDD. EPA has stated that "[b]inding to the Ah receptor appears to be necessary for all well-studied effects of dioxin but is not sufficient, in and of itself, to elicit these responses" (Draft *Dioxin Reassessment*, p. 9-78).

A number of statements by EPA (in it's Draft *Dioxin Reassessment* and in recent revisions of that document) and by the SAB (in reviewing the Draft *Dioxin Reassessment*) support the conclusion that Ah receptor binding (or even enzyme induction) does not, in and of itself, explain how adverse responses, particularly cancer, occur in animals. These statements also demonstrate that little is known about the mechanistic events leading to TCDD-induced carcinogenicity in animals and even less is known about the mechanism by which TCDD might induce toxicity in humans.

EPA states:

> In certain cases, no response occurs even when there is some receptor occupancy, a threshold phenomenon that reflects the biological "inertia" of the system (Ariens et al. 1960). In other cases a maximal response occurs well before all receptors are occupied, a phenomenon which reflects receptor "reserve" (Stephenson, 1956). Therefore, one cannot simply assume that the relationship between fractional receptor occupancy and biological response is linear. Furthermore, for a ligand (such as TCDD) that elicits multiple receptor-mediated effects, one cannot assume

> that the binding response relationship for one, simple effect (such as enzyme induction) will necessarily be identical to the binding response relationship for a different effect (such as cancer). (Draft *Dioxin Reassessment*, p. 2-5).

If the binding response relationships for enzyme induction and cancer are different, it cannot be concluded with any confidence that the mechanism of action by which TCDD-induced carcinogenicity occurs is adequately understood.

Even assuming that the mechanism of carcinogenic action of TCDD in animals involves the Ah receptor, the lack of knowledge concerning the similarities and differences between human and animal Ah receptors undermines any speculation that the mechanism for the carcinogenicity of TCDD in animals applies to humans. According to EPA, there is great uncertainty on this critical point. EPA states that "[t]he human receptor has not been studied extensively, and it is unknown if the properties of the human protein differ substantially from those of the Ah receptor in animals" (Draft *Dioxin Reassessment*, p. 2-8).

Further, EPA has concluded that "there is no clear mechanistic link between CYP1A1 induction and cancer" (Draft *Dioxin Reassessment*, p. 6-27). Because many chemicals, in addition to TCDD, can induce enzymes, the role of this event in explaining carcinogenicity is unknown (particularly for non-genotoxic chemicals like TCDD).

SAB's Review of the Draft Dioxin Reassessment Demonstrates Little is Known About the Mechanism of Action by Which TCDD Causes Cancer in Animals

The SAB's review of the Draft *Dioxin Reassessment* (SAB review) also supports the conclusion that the mechanism of TCDD-induced carcinogenicity is unknown or, at best, is poorly understood. The following excerpts from the SAB review clearly illustrate this point:

- "[M]echanisms of action for the toxic events beyond receptor binding are largely unknown" (p. 49).

- "Much of what is purported to link the Ah receptor to specific toxic events is merely the demonstration of an association between the binding of TCDD to the receptor and an eventual appearance of an adverse effect some time later in some species.... But the possible downstream events, if they exist, between Ah receptor binding and the final toxic manifestation are not well established. Mechanism of action should mean that at least some of the intermediate steps, after Ah receptor binding and leading to the pathologic processes involved, are known to some extent.... In actual fact, the only mechanism of action involving the Ah receptor that has been worked out sufficiently well to be called the biological sequence

that describes a "mechanism of action" is the induction of cytochrome P450.... The rest of the biological consequences of TCDD exposure are yet to be described adequately and sequentially in mechanistic terms" (p. 49).

- "[T]here is a large intellectual chasm between the elegant science describing the details of the TCDD receptor and its mechanism of initiating a cellular response, and the poorly understood manifestations of the toxic events associated with an alteration of the homeostasis of an animal. The linkage between Ah receptor action and specific cellular toxicity remains undefined" (p. 50).

- "Most of the "mechanistic data" support the involvement of the Ah receptor, but say little (in the context of toxicity), about how the activation of this protein alters normal physiologic function and/or development. Risk assessments based solely on Ah receptor activation or on the existing knowledge of CYP1A1 induction are unlikely to provide a biologically defensible prediction (quantitatively or qualitatively) of likely toxic outcomes in humans, particularly under low exposure scenarios" (p. 49).

- "[H]ow convincing is the evidence for the purported mechanisms that link receptor binding to toxic effects in humans? Unfortunately, the evidence is quite mixed" (p. 51).

EPA's Recent Revisions to the Draft Dioxin Reassessment

EPA's recently revised Chapter 8 of the *Dioxin Reassessment* (Dose-Response Modeling, January 27, 1997) and it's partially revised risk characterization for the *Dioxin Reassessment* (Draft *Integrated Risk Characterization for TCDD,* Sept. 24, 1996) continue to demonstrate that the mechanism of action by which TCDD might induce tumors in laboratory animals is still unknown.

EPA expresses considerable uncertainty concerning the significance of Ah receptor binding and carcinogenicity. For example, EPA acknowledges that "[t]he relationship between Ah receptor binding and carcinogenicity of TCDD is less clear" (Revised Chap. 8, p. 6). EPA also states that, "[t]he induction of CYP1A proteins are perhaps the best characterized responses to dioxins. The relevance of these proteins to the toxic effects of TCDD are controversial" (Revised Chap. 8, p. 91). EPA also states:

- "Most of the mechanistic or dose-response information on dioxin's effects has been generated on changes in gene expression of single genes such as CYP1A1 induction. There is only limited information on the complete interaction of biochemical, molecular, and biological events that are necessary to produce a frank toxic effect such as cancer..." (p. 129).

- "The development and implementation of a complete mechanistic [risk assessment] model for

the effects of TCDD identified several areas where future research is needed. Of critical utility would be data and models which are able to directly link gene expression with toxicity in a mechanistic fashion" (p. 141).

In it's Draft *Integrated Risk Characterization for TCDD,* EPA further illustrates how poorly the mechanism of action by which TCDD might induce tumors in laboratory animals is understood. EPA states:

- "The fact that TCDD **may induce** a cascade of biochemical changes in the intact animal **raises the possibility** that dioxin **might produce** a response such as cancer by mechanisms that differ among tissues....One **possible mechanism** discussed is that TCDD **might activate** a gene(s) that is directly involved in tissue proliferation. A second mechanism involves TCDD-induced changes in hormone metabolism, which **may lead** to tissue proliferation ...which **might lead** to indirect mutagenic effects. Thus, while this reassessment as identified a number of **hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity, and in which species**" (p. 72, emphasis added).

- "The ability of TCDD...to modulate a number of biochemical parameters in a species-, tissue-, and temporal specific manner is well recognized. Despite the ever expanding list of these responses over the past 20 years and the elegant work on the molecular mechanisms mediating some of these, there still exists a considerable gap between our knowledge of these changes and the degree to which they are related to the biological and toxic endpoints elicited by these chemicals" (pp. 74-5).

- "Thus, while this reassessment has identified a number of hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity and in which species" (p. 72).

- "Thus, the mechanisms by which many, if not most, of the biochemical processes are altered by TCDD treatment remain to be determined. Nevertheless, it is presumed based on the cumulative evidence available...that all of these processes are mediated by the binding of TCDD to the Ah receptor" (p. 75).

Based upon these statements, it is simply not possible to support an argument that the mechanism by which TCDD induces cancer in laboratory animals (much less in humans) is known or understood.

IARC's Recent Dioxin Review Demonstrates that Little is Known About TCDD's Mechanism of Carcinogenicity

IARC's TCDD Monograph (Appendix A to the RC Document) demonstrates that the mechanism by which TCDD induces cancer in laboratory animals is not well understood. In discussing mechanisms of carcinogenicity, IARC first admits that the precise role of Ah receptor activation in TCDD carcinogenicity "remains unclear" (IARC Monograph, p. 331). IARC then speculates concerning potential mechanisms involved in TCDD carcinogenicity. It suggests that effects on gene expression, oxidative damage, cell transformation, cell proliferation and tumor promotion, or suppression of immune surveillance could play a role. IARC, however, provides little or no support for these proposed mechanisms. For example, concerning gene expression, IARC only notes the induction of CYP1A1 and CYP1A2 and concludes that "The role, if any, of the induction of these [related]genes in carcinogenesis by TCDD is unclear." Concerning oxidative damage, IARC notes that the "relevance of these high dose studies [demonstrating oxidative damage] is questionable" (IARC Monograph, p. 332). Regarding cell transformation, IARC admits "conflicting results" (IARC Monograph, p. 340). Finally, IARC admits that the results of studies concerning the effect of TCDD on immune function in humans are inconsistent. Therefore, for each of IARC's hypotheses concerning possible mechanisms of TCDD carcinogenicity, the data are inconclusive, inconsistent, or of questionable relevance to humans.

Conclusion

Based on the totality of the available epidemiology and mechanism of action data, the most that can be concluded is that TCDD should be listed as *Reasonably Anticipated to be a Human Carcinogen*. Clearly, the available data do not support NTP's proposal to change its current listing of TCDD to *Known to Be a Human Carcinogen*.

Unlike substances on NTP's current list of known human carcinogens, the epidemiological weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria. Despite a great deal of study on numerous populations exposed to varying amounts of TCDD (some of them having extremely high exposure), the epidemiological data do not permit a conclusion that TCDD is a known human carcinogen. Indeed, three independent reviews (EPA, SAB and IARC) have concluded that the totality of the epidemiological data on TCDD

carcinogenicity are only "limited" or "suggestive." Such data do not provide "sufficient evidence" to allow NTP to classify TCDD as *Known to be a Human Carcinogen.*

Based upon numerous statements from the Draft *Dioxin Reassessment*, the SAB's comments on the Draft *Dioxin Reassessment,* and on EPA's revised versions of Chapter 8 on Dose-Response Modeling as well as the Draft *Integrated Risk Characterization for TCDD*, the mechanism of action by which TCDD induces tumors in laboratory animals is unknown or, at best, uncertain. While it is universally accepted that TCDD binds to the Ah receptor, beyond this initial event, the sequence of events leading to tumor development is essentially unknown. In fact, there are no peer reviewed publications which articulate the mechanism of action by which TCDD induces tumors in animals.

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Tab 3

CHLORINE
CHEMISTRY
COUNCIL

June 15, 1998

Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
MD EC-14, P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Jameson:

A
Council
of the
Chemical
Manufacturers
Association

The Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, is pleased to submit the enclosed comments in response to NTP's evaluation of dioxin (2,3,7,8-TCDD) for the 9th Report on Carcinogens. CCC is dedicated to addressing public policy issues related to chlorine chemistry, and therefore has a significant interest in NTP's evaluation of TCDD.

As discussed more fully in the enclosed comments, the current epidemiology data do not support reclassifying TCDD as a "known" human carcinogen. Importantly, the criteria established by the Department of Health and Humans Services (HHS) do not permit NTP to overcome this insufficiency by relying on mechanistic data to elevate TCDD to the "known" carcinogen category. However, even if such data were to be considered, TCDD's mechanism of action is not well understood.

Thank you for your consideration of these comments. If you have any questions regarding this submission, please contact Mr. David Fischer at (703) 741-5179 or Dr. Marcie Francis at (703) 741-5872..

Sincerely,

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Enclosures

Comments of the Chlorine Chemistry Council
on the National Toxicology Program's Review of 2,3,7,8-TCDD
for the Ninth Report on Carcinogens
(63 *Fed.Reg.* 18,345 - April 15, 1998)

The Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, is pleased to submit these comments in response to the National Toxicology Program's review of 2,3,7,8-TCDD for the 9th Report on Carcinogens. These comments supplement CCC's August 25, 1997 comments on NTP's proposal (attached), as well as comments presented at the October 31, 1997 public peer review meeting.

CCC applauds NTP's decision to re-review TCDD in 1998 and include another open, public review by its Board of Scientific Counselors Report on Carcinogens Subcommittee. This additional review is imperative to correct a serious procedural deficiency that occurred during the October 1997 Subcommittee review of TCDD. Recent epidemiological studies, including Hooiveld et al. (1997), became the basis of the Subcommittee's recommendation to reclassify TCDD as a known human carcinogen despite the fact that these studies were neither made available to the public nor Subcommittee members. NTP's re-review of TCDD will allow for a more thorough consideration of these epidemiological studies.

Based on the attached scientific review by Dr. Nathan Karch and Lesa Aylward of Karch & Associates, CCC continues to believe that there is insufficient evidence of TCDD's carcinogenicity in humans to indicate a causal relationship. As discussed in CCC's previous comments, the epidemiological data on populations exposed to TCDD do not constitute sufficient evidence of a causal relationship between TCDD exposure and cancer. Studies published since the October 1997 review do not change this conclusion. Importantly, the criteria established by the Department of Health and Humans Services (HHS) do not permit NTP to overcome this insufficiency by relying on mechanistic data to elevate TCDD to the "known" carcinogen category.

Even if NTP were to rely on mechanism of action data, the current data do not adequately explain the mechanism by which TCDD induces tumors in animals. Even less in known about TCDD's mechanism of action in humans. Therefore, NTP should not reclassify TCDD as a "known" human carcinogen.

NTP Improperly Applies its Own Listing Criteria for the "Known To Be A Human Carcinogen" Category

NTP has improperly relied on evidence other than human studies during its review of TCDD. Mechanism of action data is referenced in the RC Background Document and NTP's most recent call for public comments on dioxin. However, the criteria for the category of substances "known to be human carcinogens" require "sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance, or mixture and human cancer." If evidence in human studies is not sufficient to indicate a causal relationship, mechanistic data cannot be used to compensate for this deficiency.

When HHS revised the listing criteria in 1996, it did not substantively change the criteria for the "known" human carcinogen category. The notice (61 *Fed.Reg.* 50499 - September 26, 1996) shows that the only revisions made to the "known" category were the italicized editorial changes. All references to the use of mechanism of action data are under the section concerning revisions to the "reasonably anticipated to be a human carcinogen" category. The Departmental press release and an *NIEHS News* article on the criteria confirm that substantive revisions to the criteria pertain only to the "reasonably anticipated" category.

Even if Mechanistic Data is Included, the Current Data Do Not Support Reclassifying TCDD as a "Known" Human Carcinogen.

As discussed more fully in CCC's August 25, 1997 comments, TCDD's mechanism of action is not well understood. Apart from receptor binding and enzyme induction, little is known, about mechanistic events leading to TCDD induced carcinogenicity in animals. Even less is known about the mechanisms by which TCDD might induce toxicity in humans. Therefore, the available mechanistic data do not provide support for reclassifying TCDD as a known human carcinogen.

The two primary reviewers of TCDD for the October meeting supported this position. Dr. Yamasaki stated that while it is biologically plausible that TCDD could induce tumors, more direct information in exposed humans is needed. Dr. Frederick stated that the early steps of TCDD binding to the Ah receptor and to DNA response elements have been elegantly elucidated, but are not enough to explain its carcinogenicity.

Comments on the National Toxicology Program's Classification of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in the Ninth Report on Carcinogens

June 12, 1998

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Introduction

The National Toxicology Program's (NTP's) current *Biennial Report on Carcinogens* lists 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the category "Reasonably Anticipated to be a Human Carcinogen." For the ninth edition of the report, NTP is proposing to change the listing of TCDD to the category "Known to be a Human Carcinogen." Karch & Associates, Inc., is submitting these comments on behalf of the Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, in response to the *Federal Register* notice of April 15, 1998, calling for additional public comment on the proposed reclassification. These comments are a supplement to detailed written comments submitted by CCC in August and October of 1997.

Karch & Associates, Inc., contends that the science does not support this proposed change. **We base this position on NTP's stated criteria, and urge the NTP Board of Scientific Counselors to advise NTP not to list TCDD as a known human carcinogen.**

As noted in CCC's previous comments, NTP's recently revised criteria for listing chemicals as known human carcinogens requires "sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent ... and human cancer" (61 *Fed. Reg.* 50,499, September 26, 1996). As discussed in CCC's previous comments, the epidemiologic data on populations exposed to TCDD do not constitute sufficient evidence of a causal relationship between TCDD exposure and cancer. Studies published since last fall, after the last round of public comment, are discussed in some detail below. These studies do not change this conclusion.

The NTP revised criteria for classification of substances are clear. TCDD, with sufficient evidence of carcinogenicity in animals, limited mechanistic data, and limited epidemiologic data, is properly classified as "Reasonably Anticipated to be a Human Carcinogen."

Recent Epidemiologic Data Are Not Sufficient to Change the Classification of TCDD

Two relevant epidemiologic studies presenting new epidemiologic data or analyses have appeared since October 1997. Hooiveld *et al.* (1998) provides an update on the mortality experience of one of the Dutch manufacturing cohorts previously reported on by Bueno de

Mesquita *et al.* (1993). Flesch-Janys *et al.* (1998) and Becher *et al.* (1998) provide alternative exposure-response analyses on the cohort previously reported on in Flesch-Janys *et al.* (1995), Manz *et al.* (1991), and included in the four cohorts evaluated by Becher *et al.* (1996). The recent Environmental Health Perspectives Supplement (Volume 106, Supplement 2) also included a report on Seveso (Bertazzi *et al.* 1998), but the data are the same as those published in 1996 and 1997 on the 15 year mortality follow-up in this population. Also in the EHP Supplement was a report by Lynge on soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides not contaminated with TCDD. Finally, the Supplement contains a report on case-control studies that are ongoing in Vietnam, but the report does not present any results (Kramarova *et al.* 1998).

The relevant recent studies, Hooiveld *et al.* (1998) and Flesch-Janys *et al.* (1998) (with its companion, Becher *et al.* 1998), must be evaluated on their own, and also in the context of the rest of the body of epidemiology on TCDD-exposed populations. As discussed in the previous CCC comments, this body of literature suffers from confounding by exposures to a wide variety of industrial chemicals (many of which are known to be human carcinogens or are potential carcinogens) and smoking. This confounding is evident from the inconsistency and lack of specificity in the tumor categories reported to be elevated. Different studies report elevations in different cancers, and report only marginally elevated SMRs in cohorts exposed to very high levels of TCDD.

An example of the problems of confounding can be found in the study of the U.S. manufacturing cohorts by Fingerhut *et al.* (1991). Fingerhut *et al.* (1991) is often cited as the strongest of the previous studies, with portions of this cohort exposed to TCDD at levels 1,000 to 10,000 times background levels. Yet, the SMRs for total cancers and lung cancer reported in this study were less than 1.5, and the cohort was exposed to numerous other chemicals, including known carcinogens, in the workplace. There was no trend in SMRs for either lung or total cancers with duration of employment in exposed job categories, and there was substantial evidence of confounding by smoking. The authors attempted to address the potential for confounding by smoking by surveying smoking status in a non-random sample of individuals, taken from two of the twelve manufacturing cohorts included in the study. The authors

concluded that although the smoking habit was somewhat elevated over the general population in this sample:

> "...the increased number of lung cancers in the high exposure subcohort was probably not due to confounding by smoking, for several reasons. First, other diseases related to smoking were not more common than expected in this subcohort; mortality from nonmalignant respiratory disease, which is often associated with smoking, was lower than expected ..."

However, in a detailed analysis of the Fingerhut *et al.* (1991) study and data, Delzell *et al.* (1994) found that deaths from nonmalignant respiratory diseases *were* significantly elevated in the largest plant included in the cohort. This plant contributed 67 percent of the lung cancer cases observed in the high exposure subcohort, but only 8 percent of all deaths from cancer. Workers at this plant were exposed to approximately 150 different chemicals. Delzell *et al.* (1994) note that in another study of the workers from this plant, lung cancer mortality was elevated even among the workers never assigned to a TCDD-contaminated process. Finally, examination of data from the different plants included in the Fingerhut *et al.* (1991) study and by period of hire cast further doubt on a causal relationship between TCDD exposure and the observed excess of lung cancer. In particular, lung cancer and total cancers each were statistically significantly elevated at only one of the twelve plants included in the study (the plant discussed above, where elevations in lung cancer were observed even in workers not exposed to TCDD). Since the excess in respiratory cancer (and presumably, the occurrence of other smoking-related cancers and cancers due to other chemical exposures at this plant) strongly influenced the finding of elevated total cancer mortality in Fingerhut *et al.* (1991), the findings of elevations in all cancers and of lung cancer in this study cannot be reliably attributed to exposure to TCDD.

Similarly, the recent publication of the IARC cohort analysis (Kogevinas *et al.* 1997) seemed to indicate a small excess of total cancer and lung cancer (SMRs for each of 1.12). However, the results of this study were strongly influenced by the inclusion of the NIOSH

cohort. When the NIOSH cohort members were excluded from the study, the observed excess in total cancers was no longer statistically significant.

Other specific cancers were found to be somewhat elevated in other previous studies, but the findings have been highly inconsistent from one cohort to another.

- Saracci *et al.* (1991) reported excesses in thyroid and stomach cancer, but no excesses in these sites were observed in other studies.

- Bertazzi *et al.* (1996, 1997) reported on the Seveso population exposed to TCDD after an accident. Fifteen years after the accident, no increase in total cancer mortality was observed in any of the three subcohorts (Zone A, highest exposure; Zone B, medium exposure; Zone R, low exposure). Elevations have been observed in males from Zone B for rectal cancer and leukemia and in Zone R for cancer of the esophagus. In females in Zone A, excesses in deaths from melanoma and "other digestive" cancers were observed, based on 1 and 2 deaths, respectively. Also in females, in Zone B, deaths from myeloma and Hodgkin's disease were elevated, and the elevation in deaths from brain cancer was borderline statistically significant (based on 3 cases). However, the pattern is not consistent either among exposure subcohorts within the Seveso population (no excesses were observed in males in Zone A, and cancers elevated in Zone R were not elevated in Zone B, while cancers elevated in Zone B were not elevated in Zone A), or with excesses seen in other studies (for example, no excess in respiratory cancers was observed).

- The most recent update of the Ranch Hand mortality study (Ketchum and Akhtar 1996), covering mortality experience through December 31, 1993, found no increases in total mortality, cancer mortality, or mortality from cancer of any site after more than twenty years of follow-up. There was no evidence of increasing risk with increasing body burden of TCDD.

- Ott and Zober (1996), studying a small (N=243) cohort exposed to TCDD during and after an accident at a manufacturing facility in Germany, found an increase in total cancer mortality correlated with TCDD level, but only among smokers; no trend was found among non-smokers. Smoking was found to be highly significantly correlated to total cancer mortality in this cohort, while correlation with TCDD exposure was borderline significant in the cohort. This pattern suggests confounding by smoking in this cohort.

Finally, when positive associations have been reported in studies of TCDD-exposed populations, they have been of very low magnitude. Reported SMRs for total cancer mortality have generally been less than 1.5; Kogevinas *et al.* (1997) reported an SMR of 1.12. This low degree of elevation is especially striking in light of exposures in these cohorts that may be as much as 10,000 times background levels, comparable to levels leading to large elevations in tumors in animals (Kociba *et al.* 1978). It is very difficult to rule out confounding or bias as contributing to elevations of such low magnitude, even if the elevations are statistically significant. Assessments of statistical significance provide an assessment of the likelihood of the finding occurring by random variations, but cannot account for confounding or bias. This issue was discussed in the context of observational epidemiology in a recent *Science* article (Taubes 1995), in which noted epidemiologists discussed the problems associated with interpreting relative risks of less than two or three in a particular study. Even when a small increase in relative risk is found in several studies of similar design, David Sackett from Oxford University noted that "if there's an inherent bias, it wouldn't make any difference how many times it's replicated."

It is against this background of inconsistent, weak, and confounded results that the recent epidemiologic studies, Flesch-Janys *et al.* (1998) and Hooiveld (1998) must be evaluated.

Flesch-Janys et al. (1998)

Flesch-Janys *et al.* (1998) present a dose-response analysis within the Boehringer-Ingelheim cohort, using an estimated area-under-the-curve (AUC) of serum lipid TCDD levels and TEQ AUC as the dose measures. The cohort was divided into quartiles based on the

estimated AUC values, and response was reported as SMRs based on the general German population. The cohort as a whole had statistically significantly elevated SMRs for all causes (SMR 1.15; 95% C.I. 1.05-1.27), total malignancies (SMR 1.41; 95% C.I. 1.17-1.68), cancer of the rectum (SMR 2.30; 95% C.I. 1.05-4.37), lung cancer (SMR 1.51; 95% C.I. 1.07-2.08), respiratory cancers including lung cancer (SMR 1.71; 95% C.I. 1.24-2.29), and lymphatic and hematopoietic cancer (SMR 2.16; 95% C.I. 1.11-3.77).

The authors then performed an analysis by exposure quartile for death from all causes, total cancers, lung cancer, and lymphatic/hematopoietic cancer. No trend by increasing TCDD exposure level was seen for death from all causes, lung cancer, or lymphatic/hematopoietic cancers. For each of these, the SMR for the lowest-exposed quartile was essentially identical to that for the highest-exposed quartile, with the middle exposure groups displaying lower SMR values. None of the quartile-specific SMR values were statistically significant for these analyses. The trend for total cancer SMR by quartile was statistically significant. However, of the individual SMR values for quartiles I through IV (1.24, 1.34, 1.34, 1.73, respectively), only the value for the highest quartile was statistically significant, and, although estimated exposure increased by more than a factor of twenty, the observed increase in SMR was slight.

The cohort examined in Flesch-Janys *et al.* (1998) was exposed to numerous chemicals as part of their employment, including several known or suspected carcinogens. Chemical exposures included benzene (a known human carcinogen producing hematopoietic cancers), dimethyl sulfate, lindane and hexachlorocyclohexane (see Flesch-Janys *et al.* 1995). The SMR values observed in the trend analyses for the lowest-exposed quartile, which were similar to those for the highest-exposed quartile, indicate that the cohort as a whole is experiencing slightly elevated mortality from cancers *independent* of level of exposure to TCDD, indicating that there is confounding in this study. Thus, the results of this study do not change the limited nature of the epidemiologic evidence as a whole.

A related analysis by Becher *et al.* (1998) used the same exposure data and cohort with a relative risk analysis to examine the shape of possible dose-response curves. The cohort was divided into six exposure categories, and relative risk was assessed comparing total cancer mortality in each exposure group to the lowest exposed group. Although the trend in relative risk

values was statistically significant, none of the individual dose group relative risks were statistically significant.

On a separate issue, the analysis in Flesch-Janys *et al.* (1998) provides information related to the widespread assumption that Ah receptor binding is directly related to tumor induction. Exposure analysis using TCDD Toxicity Equivalence values (TEQ) assesses total "TCDD-equivalent" exposure based on the relative potencies for Ah receptor binding and enzyme induction of various dioxin and furan congeners compared to TCDD. The trend analysis in Flesch-Janys *et al.* (1998) using TEQ AUC revealed no relationship between TEQ AUC and SMR values for any of the endpoints examined, including total cancer mortality. If Ah receptor binding were directly related to tumor induction, a dose-response analysis using TEQ exposure should result in a stronger dose-response relationship than using TCDD alone. However, the opposite was the case in this study. Thus, this observation undercuts any assumption that Ah receptor binding and resulting enzyme induction, which is the primary basis for TEQ values for dioxin and furan congeners, has any direct mechanistic relationship with tumor development.

Hooiveld et al. (1998)

The recent study by Hooiveld *et al.* (1998) is seriously flawed. Hooiveld *et al.* (1998) presents an update on the mortality experience of the cohort from a facility designated as "Factory A" in Bueno de Mesquita *et al.* (1993). Of the 1,031 male workers in the cohort, 549 were classified as "exposed" to TCDD and 482 were considered to be unexposed. The study presents both an SMR analysis of the TCDD-exposed cohort as a whole, and a relative risk analysis comparing the TCDD-exposed portion of the cohort to the unexposed portion. In addition, the authors attempted to construct an exposure index, and used the exposure classification to compare workers with "high" and "medium" exposure to those with "low" exposure.

The SMR analysis revealed a small elevation in SMR for death from all cancers combined (SMR 1.5; 95% C.I. 1.1-1.9). Kidney cancer was also statistically significantly elevated based on four cases (SMR 4.1; 95% C.I. 1.1-10.4). The relative risk analysis, comparing the cancer mortality in the TCDD-exposed portion of the population with the

unexposed group, yielded a much higher relative risk for total cancers (SMR 4.1; 95% C.I. 1.8-9.0). However, this analysis is seriously flawed.

The unexposed portion of the population is much younger than the exposed population. Although the authors do not discuss this directly, this can be seen from examination of the data in Table 1 in Hooiveld *et al.* (1998). The year of first exposure and time since first exposure clearly shows that the exposed group is a much older population. This is confirmed by examining the percentage of each subcohort that has died: 25 percent of the exposed subcohort had died at the time of the study, versus only 8 percent of the unexposed subcohort. The authors state that the relative risk analysis was "adjusted for age and calendar year at end of follow-up and time since first exposure/employment." However, statistical adjusting cannot account adequately for fundamental differences in the population demographics. When the relative risk analysis is examined with this in mind, comparison of the two groups will inevitably lead to misleading results. Only 7 cases of death from malignant neoplasms occurred in the unexposed subcohort. There is bound to be a dearth of cancers that occur primarily at older ages in the unexposed group regardless of exposure and any role of TCDD in cancer causation, and there is no way to "adjust" for this flaw. In fact, the authors had to insert a fictitious death from urinary tract cancer in the unexposed subcohort to make the computer program converge. There are too few members of comparable birth cohorts in the unexposed population for them ever to serve as an appropriate reference population for the much older exposed population.

The authors went further in their relative risk analysis, by dividing the entire cohort (both "exposed" and "unexposed") into "high," "medium," and "low" exposure categories. In practice, this resulted in the "low" exposed group consisting of the "unexposed" group from the previous analysis along with 48 people from the TCDD-exposed group judged to have low exposure. The remaining exposed cohort members were divided into those with estimated maximum TCDD serum lipid levels from 7.7 to 124.1 ppt (medium exposure) and those with estimated maximum serum lipid TCDD levels from 124.2 to 7,307.5 ppt. Despite the difference in exposure levels between the medium and high exposure groups (up to a factor of nearly 100), the relative risk for total cancer in the high exposure group was lower than that observed in the medium exposure

group (medium exposure group SMR 4.8; 95% C.I. 2.0-11.3; high exposure group SMR 4.4, 95% C.I. 1.9-10.4). These data provide no evidence of a dose-response.

Finally, the magnitude of the supposedly statistically significant relative risks reported in this analysis (a relative risk of more than 4 for total cancer using the younger unexposed cohort as the reference population) compared to the weak increase in SMR in the identical cohort (using the general Dutch population as the reference population) points out the problem with using the much younger unexposed population as the reference population in this analysis. Whatever potential advantage exists in using an internal reference population that is presumed to match the exposed subcohort better than the general population is undermined by the difference in age distribution in the internal reference population in this study. The relative risk analysis in this paper is not informative, and certainly does not constitute strong support for a causative role of TCDD exposure in any cancer excess observed in this cohort.

Although it is not discussed in Hooiveld *et al.* (1998), the workers at this plant were involved in the formulation and synthesis of dozens of pesticides. Table 1 of Bueno de Mesquita *et al.* (1993) lists at least 60 different pesticides and compounds, including DDT, sodium arsenite, hexachlorocyclohexane, lindane, and toxaphene. In addition, other chemical agents that were present in the work environment included numerous solvents, amine compounds, mono- and tri-chlorobenzene, and other chemicals. A role for any of these chemicals, or other sources of confounding such as smoking habits, cannot be ruled out in the search for the underlying cause of the slightly elevated SMR observed in the "exposed" cohort as a whole. Attribution of this elevation to exposure to TCDD is not scientifically supportable. The Hooiveld *et al.* (1998) study provides no compelling data to change the overall assessment of the epidemiologic data on TCDD as limited.

Role of mechanism

NTP's revised criteria do not specifically allow for consideration of mechanistic data in the classification of a substance as a known human carcinogen. However, even if mechanistic data were to be considered in this evaluation, it would not result in a change of classification for TCDD. Most scientists believe that the mechanism of action for TCDD-induced toxicity

(presumably including carcinogenesis) is based on an initial step of TCDD binding to the Ah receptor, but no further insight into the mechanism of action of TCDD-induced animal carcinogenesis exists, despite intensive research in this area. These issues were discussed recently at the Receptor Biology Roundtable involving leading academic, industrial, and regulatory agency scientists organized by Dr. William Greenlee of the University of Massachusetts Medical School and Dr. Arman H. Tashjiam, Jr., of the Harvard School of Public Health (Limbird and Taylor 1998). Thus, evidence of similarity of Ah receptors in humans and animals, or even of events known to be a consequence of binding to the Ah receptor, such as enzyme induction, do not provide support for classification of TCDD as a known human carcinogen. The events that lead to tumor induction in animals still remain to be elucidated.

Conclusions

There are at least two alternative explanations to the small elevations in total cancers observed in various occupational populations, with underlying excesses in different tumors in different populations. One explanation is that exposure to other agents present in the occupational environment as well as smoking and other lifestyle habits resulted in excesses in various cancers and overall cancer rates in these populations. A second explanation is that TCDD exposure caused the observed elevations. Given the presence of known carcinogens in most, if not all the occupational cohorts in which elevations have been observed, and given the lack of such generalized elevations in other populations with fewer confounding exposures (Seveso, Ranch Hand), the alternative exposures and causes must be systematically evaluated before the observed generalized excesses can be credibly attributed to TCDD exposure.

Unlike substances on NTP's current list of *Known Human Carcinogens*, the epidemiologic data for TCDD does not fulfill well-recognized causation criteria. The recent studies published since CCC submitted comments in August and October of 1997 do not change the weight of the evidence, and the conclusions of other recent reviews by IARC, EPA, and the EPA's Science Advisory Board (SAB) are still appropriate: the epidemiologic data on TCDD are "suggestive" or "limited." The data for TCDD clearly place it in the category "Reasonably

Anticipated to be a Human Carcinogen." We urge the Board of Scientific Counselors to maintain the current, appropriate classification.

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Tab 4

Comments of Nathan J. Karch, Ph.D., D.A.B.T. on behalf of The Vinyl Institute on the National Toxicology Program's Classification of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin for the *Ninth Report on Carcinogens*

November 24, 1998

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Introduction

The National Toxicology Program's (NTP's) current *Biennial Report on Carcinogens* lists 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the category "Reasonably Anticipated to be a Human Carcinogen." For the ninth edition of the report, NTP is proposing to change the listing of TCDD to the category "Known to be a Human Carcinogen." Karch & Associates, Inc., is submitting these comments on behalf of The Vinyl Institute.

The science does not support this proposed change because the human epidemiology is limited and our understanding of mechanism is far too incomplete to override the human evidence. **Therefore, we urge the NTP Board of Scientific Counselors to advise NTP not to list TCDD as a known human carcinogen.**

Criteria

The subcommittee is deciding whether the data on TCDD fit the previous characterization of TCDD as "Reasonably Anticipated to be a Human Carcinogen" or whether the evidence justifies a reclassification to a characterization of "Known Human Carcinogen." According to NTP's own criteria, placing a substance in the category of "Known Human Carcinogen" requires sufficient evidence from human studies to conclude that a causal relationship exists. Good science dictates that categorizing TCDD or any other chemical as a "Known Human Carcinogen" requires that the evidence be strong, clear, consistent, coherent, and distinguishable from chance, bias, and confounding. The epidemiology is insufficient to support the proposed reclassification of TCDD.

Epidemiologic Evidence is Limited

Several bodies, including IARC, EPA, and the EPA Science Advisory Board, and individual authors as well (e.g., Neubert 1998), have concluded that the human epidemiologic evidence is "limited" or "suggestive," but not "sufficient." In written comments that I submitted to NTP in June on this issue (Karch and Aylward 1998), I discussed the epidemiologic evidence in detail. I noted that it is weak and inconsistent, with small elevations in total tumors due to excesses in different underlying tumors being reported in industrially-exposed populations. These studies, with SMRs generally less than two, and often under 1.5, do not adequately address issues of potential bias and confounding. The populations studied all have potentially confounding exposures or lifestyle factors that have not been adequately accounted for in the study designs. Table 1 summarizes some of the confounding factors or exposures present in some of the populations studied. In the face of these confounding exposures, many of which would be highly correlated with TCDD exposure, attribution of the varying small observed excess cancer rates to TCDD is unwarranted.

Understanding of Mechanism is Insufficient to Justify Proposed Reclassification

Despite its own classification criteria, NTP in its background document, which relies on the IARC monograph, cites an understanding of mechanism as pivotal in upgrading the classification of TCDD. If mechanism is to be used to override the limited nature of the human epidemiologic evidence and NTP's own criteria to justify the elevation of the classification of TCDD to that of a "Known Human Carcinogen," the understanding of mechanism must be clear and complete. It is neither.

Rather than delineating the actual steps in mechanism, IARC and NTP simply cite a role for the Ah receptor and its conservation in mammalian species as the main mechanistic information central to extrapolating animal findings to humans. IARC states:

> 2,3,7,8-TCDD is a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor; this receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals... (p. 343).

However, principal reliance on the role of the Ah receptor as a basis for a mechanism common to humans and animals is overly simplistic and a misrepresentation of the state of the science. The array of recent mechanistic research highlights the inadequacy of Ah receptor binding as a mechanism of action for TCDD-induced carcinogenesis in animals, much less in humans.

Understanding of Mechanism of Carcinogenesis is Limited

Binding to the Ah receptor may be the initial step in the mechanism of tumorigenesis. However, while theories abound, the link between tumor production and Ah receptor binding with nuclear translocation of the ligand-receptor complex is not well understood and may not be direct. Modulation of gene expression subsequent to binding of the ligand-receptor complex is cited as the next step in mechanism, and numerous candidates for the critical genes and gene products have been proposed and are being researched, included growth factor receptors, cell cycle regulators, and others (reviewed in Safe 1995). However, none of these endpoints is so well understood that we can say that we understand how rat liver tumors form, much less how tumors in other tissues or species might occur. Differences from tissue to tissue and from species to species in presence or absence of particular genes, and differences in the kinetics of their expression or interactions with other factors, will greatly affect the likelihood of tumor production.

Even if the Ah receptor is involved in tumorigenesis, the full sequence of steps from binding to the receptor to tumor production is not known. In contrast to this lack of knowledge, we have a full understanding of the steps from binding of TCDD to the Ah receptor leading to enzyme induction. Induction of cytochrome P450 1A enzyme activity

is a well-understood and direct result of Ah receptor binding in animal tissues. However, what happens downstream in the mechanism from binding to the Ah receptor leading to the production of tumors is not well understood, even in the extensively-studied rat liver tissue. NIEHS researchers who have studied enzyme induction and tumorigenesis in rats contrast the dose-response relationships between the two responses:

> Of particular interest is our finding that the dose response relationships for CYP 1A1 and CYP 1A2 induction are considerably different than those for TCDD-mediated increases in cell proliferation and preneoplastic foci...It is likely that the ED_{50} [for cell proliferation] is at least an order of magnitude higher than for enzyme induction. Furthermore, there may be a threshold dose for TCDD-mediated increases in cell proliferation and preneoplastic foci. (Lucier et al. 1992).

Recent research from this group demonstrates the reversibility and concentration and time dependence of cell proliferation events in the rat liver (Walker et al. 1998). The complex dose-time-response relationship for cellular proliferation in rat liver tissue (always in the presence of significant liver tissue toxicity) demonstrates that if binding to the Ah receptor is a necessary step in TCDD-induced tumorigenesis, it clearly is not sufficient. Factors other than Ah receptor binding must play critical roles in tumor induction, but what those factors are, and possible tissue and species variations in them, are not yet understood.

I also find evaluation of the enzyme induction response useful in evaluating the biological significance of human TCDD exposures. Researchers were unable to detect a relationship between markers for enzyme induction and TCDD serum level in a subset of the NIOSH cohort, including persons highly exposed to TCDD. Of particular note, the effects of smoking and alcohol consumption were clearly evident (Halperin et al. 1995). Thus, human exposures to TCDD in this cohort were apparently insufficient to produce enzyme induction. If the direct sentinel effect of Ah receptor binding, enzyme induction, cannot be detected in this group, it is unlikely that tumorigenesis due to binding of TCDD to the Ah receptor has occurred in this group or others similarly exposed.

Tumor production may involve mechanisms independent of Ah receptor binding in animals exposed to TCDD. The IARC review and the NTP background document acknowledge that several potentially Ah receptor-independent mechanisms are being investigated, including modulation of apoptosis in transformed cells, alteration of cellular gap junctions, and modulation of protein phosphorylation. Research in each of these areas is active and ongoing. Therefore, overriding the limited human evidence based on the assumption of an Ah-receptor dependent mechanism to upgrade the classification of TCDD is clearly wrong.

Our understanding of mechanism is so incomplete that, not only do we not understand the details of the steps in tumor induction, we also do not understand the mechanism for the anticarcinogenic effects of TCDD observed at low doses. The Kociba et al. (1978) rat bioassay demonstrated a significant reduction in several tumor types and in total tumor

burden at all doses tested. In particular, tumors in estrogen-responsive tissues were reduced. *In vivo* and *in vitro* results in animals support this conclusion, and results from the Seveso population appear to support this trend in humans (data reviewed in detail in Safe 1995). In the two stage rat liver model, TCDD treatment after administration of an initiator reduces the number and volume of altered hepatic foci at lower doses (see, for example, Pitot 1987). Based on the experimental evidence, TCDD is more likely to act as an **anticarcinogen** than a carcinogen in humans at doses encountered in the environment, and perhaps even at doses in occupational settings. Our understanding of mechanism is clearly too limited to override the limited human epidemiologic evidence and upgrade the classification of TCDD.

Interspecies Differences are Substantial

Great differences exist between animals and humans in how the body handles TCDD. This is reflected in differences in half-life, distribution in various organs, and in the striking qualitative and quantitative differences in toxicity among animal species and between humans and animals. Toxicity is believed to be the result of changes in gene expression for numerous proteins and factors. The genetics and kinetics of expression of these factors are likely to vary among tissues and among species; thus differences in toxicity should not be unexpected. These differences in toxic responses highlight the peril in over-interpreting our limited understanding of the consequences of binding to the Ah receptor and the other factors and events that may be necessary to elicit toxicity in general, and carcinogenicity in particular, in animals or in humans.

Conclusion

Our lack of understanding of the details of mechanism and the validity of extrapolation between tissues and species is obvious. Given

- the lack of detailed understanding regarding mechanisms of tumorigenesis even in rat liver tissue;
- the likelihood that numerous gene products and other factors not yet understood play critical roles in tumorigenesis in the rat; and
- the active research occurring into mechanisms that may be independent of the Ah receptor,

it is a gross oversimplification to state that we understand the mechanism of carcinogenicity.

Our current level of understanding of the mechanism for tumor induction does not justify overriding the limited nature of the human epidemiologic evidence. The data for TCDD clearly place it in the category "Reasonably Anticipated to be a Human Carcinogen." We urge the Board of Scientific Counselors to maintain the current, appropriate classification.

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Table 1: Confounding Exposures for Some TCDD-Exposed Cohorts

Population	Known Confounding Exposures	References
NIOSH (U.S.)	Over 150 chemical exposures at Plant 10, which accounted for 67 percent of the excess lung cancers, and in which lung cancer was reported to be elevated even in workers not exposed to TCDD Smoking	Delzell et al. 1994 Fingerhut et al. 1991
Boehringer-Ingelheim (Germany)	Benzene Dimethyl sulfate Lindane Hexachlorocyclohexane Smoking	Flesch-Janys et al. 1998
Netherlands	DDT Sodium arsenite Hexachlorocyclohexane Lindane Toxaphene Solvents Amine compounds Mono- and Tri-chlorobenzene More than 60 other industrial chemicals and pesticides Smoking	Bueno de Mesquito et al. 1993

Tab 5

67 Legare Street, #403
Charleston, SC 29401
November 23, 1998

Dr. Larry Hart
Executive Secretary
National Toxicology Program
Board of Scientific Counselors
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Hart:

More than a decade ago, I served as a principal investigator of a CDC-sponsored multi-center study of cancer in relation to potential military exposure to Agent Orange. Since that time, I have remained involved with epidemiologic research on human health in relation to exposure to dioxin-like compounds. That involvement included service as an invited observer of the 1997 IARC working group evaluation of the carcinogenicity of dioxin. Last year, I prepared written comments for the NTP Board of Scientific Counselors' Report on Carcinogens Subcommittee and made a brief oral presentation at the Subcommittee meeting.

The Chlorine Chemistry Council has asked me to provide the Subcommittee with an updated independent scientific evaluation of this topic. The attached comments are submitted to the Subcommittee in order to help clarify issues related to the 1997 IARC working group evaluation and the epidemiologic literature that has been published subsequently. This critique is based upon my own personal judgment as a cancer epidemiologist, provost of an academic health science center, and former public health dean.

I hope that the Subcommittee will find these comments to be helpful. I plan to attend the upcoming meeting of the Subcommittee and would be grateful for the opportunity to make a few remarks to the Subcommittee at that time. Thanks in advance for your consideration.

Sincerely,

Raymond S. Greenberg, MD, PhD

Enclosure

Comments on: RC Draft Background Document for TCDD and Supplement

Raymond S. Greenberg, MD, PhD
Charleston, SC

Executive Summary

In 1997, the International Agency for Research on Cancer (IARC) reviewed the scientific evidence on the potential carcinogenicity of dioxins. That review concluded that: "There is *limited evidence* in humans for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD)" (p. 342). The IARC Working Group found that the epidemiologic evidence was not sufficient to establish causality for several reasons. First, strength of exposure-disease relationships is one of the principal criteria for judging causality in epidemiologic studies. The statistical associations between exposure to TCDD and risk of cancer are very weak. Second, the cancer outcome most consistently linked to TCDD exposure in these studies is the aggregation of all cancers combined. There is little precedent in epidemiology for a causal risk factor to be associated with all types of cancer combined, without stronger associations to particular types of cancer. Third, confounding by other exposures could not be excluded from some of the observed associations. Although additional epidemiologic studies of this topic have appeared in the peer-reviewed literature since the time of the IARC review, the key data from virtually all of these reports were published in preliminary form prior to the IARC review and were cited in the IARC document. The small amount of truly new epidemiologic information that has appeared since the IARC review does not support any change in the classification of this compound with respect to carcinogenicity.

A. Comments on the Draft Background Document:

1. The Draft Background Document misrepresents one of the most important conclusions of the IARC Working Group. On p. RC-1, lines 17-18, the Background Document states that: "In the highly exposed industrial sub-cohorts, a *causal* (my emphasis) relationship between TCDD exposure and mortality from all cancers combined was noted . . ." The Background Document later reiterates this claim on p. 3-2, lines 6-8: "In its summary of the epidemiological evidence for the most highly exposed populations, the IARC Working Group identified a *causal* (again, my emphasis) association between TCDD exposure and all cancer combined . . ." In actuality, the IARC report clearly stated that the "lack of precedent for a multi-site carcinogen without particular sites predominating means that the epidemiological findings must be treated with caution" (p. 337). Most importantly, the IARC Working Group concluded that "there is *limited evidence* in humans for the carcinogenicity" of TCDD (p. 342).

2. The Draft Background Document overstates the extent to which literature published since the IARC Working Group report provides new information. For instance on p. RC-1, lines 19-23, the Background Document indicates that: "Increased risk for certain cancers was also reported in a *new* (my emphasis) study of the Seveso, Italy, dioxin-exposed population (Bertazzi et al., 1998 [in press]). These additional findings were not considered in the IARC evaluation and further strengthen the association between dioxin-exposure and human cancer." The paper in question was not a *new* study. Rather, it was an update of a cohort that has been followed since 1976. Findings from this study have appeared multiple times in the peer-reviewed literature as cited in the report of the IARC Working Group (Bertazzi et al., *Am. J. Epidemiol.*, 1989;**129**:1187-99 and Bertazzi et al., *Organohalogen Compounds* 1996;**30**:294-6). The so-called 'new' findings were presented in Table 33 of the IARC report (pp. 146-7) and in the corresponding text (p. 161). The only aspect of the 'new' findings that was not included in the IARC report was a subanalysis by latency. The IARC Working Group was aware of these results, but did not include them in the report because they had not been accepted yet for publication in the peer-reviewed literature.

3. The Draft Background Document does not provide a balanced discussion of whether dose of exposure to TCDD appears to be related to risk of cancer occurrence in humans. On p. 3-2, Lines 2-3, the Document indicates that: "In the largest and most heavily exposed German cohort, a dose-response relationship was noted for overall cancer mortality." The Draft Background Document fails to note that dioxin measurements were made on only 15% of this cohort and that the sampled individuals were certainly not a random subset of the entire cohort. Equally important, the Draft Background Document does not mention that the response was flat across all levels of dose except for a single outlying elevation in the highest exposure category. The authors inappropriately assessed the statistical significance of the relationship with a test for linear trend, despite the fact that the underlying risk pattern clearly was not linear. Finally, the citation for this dose-response relationship is a published erratum (Flesch-Janys et al., *Am. J. Epidemiol.*, 1996;**144**:716). In their original calculations, the authors used an incorrect follow-up date, which led to an overestimation of relative risks and overstatement of the statistical significance of individual point estimates. The history of computational inaccuracies in such important data does not instill confidence in these findings.

B. Comments on the Supplement:

Section 2 of the Supplement briefly describes various updates of epidemiologic studies published during 1997 and 1998 and Section 3 provides a bibliography of these references. Of the ten references cited, five appeared in a supplement to *Environmental Health Perspectives*, and are the written versions of invited talks at a November 1996 meeting at the German Cancer Research Center. This meeting in

Heidelberg preceded the IARC Working Group meeting by four months. One of the IARC epidemiology panel members (Dr. Becher) co-hosted the Heidelberg meeting and several of the other epidemiology panel members attended the Heidelberg meeting, including three who presented papers there (Drs. Becher, Bertazzi and Kogevinas). The results presented in these five supplementary papers, therefore, were well known to the members of the IARC Working Group.

1. Previously Highlighted Supplementary Reports

Of the ten cited supplementary references, two were given special attention in either the Draft Background Document or the meeting of the Report on Carcinogens Subcommittee in October 1997.

a) Bertazzi et al., *Epidemiol.*, 1997;8:646-52.

The Draft Background Document makes repeated reference to the update of the Seveso cohort, which at that time was in press and subsequently, published (Bertazzi et al., *Epidemiol.*, 1997;**8**:646-52). Last year, I provided comments on this paper for the benefit of the Subcommittee. The essence of this critique is summarized below, with a complete copy of my earlier comments reproduced as an appendix to this current submission.

i) A large number of separate analyses were conducted by Bertazzi et al., stratified by exposure zone, gender, and cancer type. Of the 159 outcomes, eight reached statistical significance at the 0.05 level; this is exactly the number of positive associations that one would have expected by chance alone.

ii) The results reported by Bertazzi et al. lacked internal consistency. For example, in zone B apparent excesses of respiratory cancer and leukemia were observed among males, with apparent deficits for these same sites among females.

iii) The pattern of positive results reported by Bertazzi et al. is random, with no apparent dose-response gradient. In the highest exposure zone for example, males actually experienced less than half of the cancers that were expected. For digestive cancer, a grouping highlighted by the authors as potentially associated with exposure, males in the highest exposure zone experienced a borderline statistically significant 80% deficit.

iv) The findings reported by Bertazzi et al. are largely inconsistent with other studies of human populations heavily exposed to dioxin. Aggregating results across exposure zones and genders, no excesses were observed for all cancers combined (Relative Risk

= 0.93), lung cancer (Relative Risk = 0.95), non-Hodgkin's lymphoma (Relative Risk = 0.92), or soft tissue sarcoma (Relative Risk = 1.0).

v) By combining all digestive disease cancers into a single site, Bertazzi et al. used an unconventional classification. This aggregation includes sites as diverse as the pancreas, liver, stomach, colon, and rectum. These sites are not only distinct anatomically, histologically and physiologically; they also have markedly different epidemiologic risk factor profiles. Therefore, it makes little sense from an epidemiologic perspective to treat them as a single category.

vi) Latency analyses were presented by Bertazzi et al. only for selected anatomic sites and for only one exposure zone. No tests of statistical significance were performed. As in other analyses, a lack of consistency across genders also was observed for latency.

vii) The possibility of confounding was not thoroughly explored by Bertazzi et al. For example, three pleural cancers were observed for males in zone B (a fivefold increase), but no attempt was made to assess the possible role of asbestos exposure.

b. Hooiveld et al., *Am. J. Epidemiol.*, 1998;147:891-901. At the October 1997 Report on Carcinogens Subcommittee meeting, Dr. Arnold Schecter was invited to present to the Subcommittee the rationale for listing TCDD as a known human carcinogen. In his presentation, Dr. Schecter included data from a report by Hooiveld et al. that was not published in the peer-reviewed literature at the time and was not included in the Draft Background Document. In the interim, the paper by Hooiveld et al. was published (*Am. J. Epidemiol.*, 1998;147:891-901). In June 1998, I provided the Subcommittee with some written comments on the paper by Hooiveld et al. The essence of my critique is summarized below, with a complete copy of my earlier comments reproduced as an appendix to this current submission.

i) Although Dr. Schecter implied that the results reported by Hooiveld et al. were new additions to the literature, the main findings of this paper were published previously (*Organohalogen Compounds* 1996;30:185-9) and this population was one of four industrial cohorts given particular emphasis in the IARC monograph (Table 38).

ii) The findings presented by Hooiveld et al. revealed a generalized increase in mortality, without any clear specificity with particular outcomes. This lack of specific exposure-disease relationships violates one of the basic tenets of causal reasoning in epidemiology and raises the possibility of a systematic error in the comparison of observed with expected mortality.

iii) The findings reported by Hooiveld et al. reveal inconsistencies with those reported in other cohorts. For example, the Dutch cohort suggests high elevations in risk of bladder and renal cancer that are not seen in other dioxin-exposed industrial cohorts.

This type of inconsistency suggests the possibility that the Dutch workers were exposed to other carcinogens.

iv) The results presented by Hooiveld et al. concerning back-calculated TCDD levels must be viewed with particular caution. Only 4% of the cohort had serum measurements obtained, and two-thirds of those selected for blood sampling did not have useable results.

v) The dose-response relationships presented by Hooiveld et al. do not demonstrate a clear gradient of effect. For example, the risk of urinary cancers rose from low to medium exposure, only to fall back to baseline in the highest exposure group. For all cancers combined and for lung and respiratory cancer, no increase in risk was observed between the medium and highest exposure groups.

2. Other Supplementary Reports:

a) **Becher and Flesch-Janys, *Environ. Health Perspect.,* 1998;106(Suppl. 2):623-4.** This is an overview of the November 1996 symposium hosted by the German Cancer Research Center. It is a summary of the meeting and provides no additional data.

b) **Becher et al., *Environ. Health Perspect.,* 1998;106(Suppl. 2):663-70.** This publication derives from the November 1996 symposium hosted by the German Cancer Research Center. It provides a reanalysis of the dose-response relationship that was published previously (*Am. J. Epidemiol.,* 1995;**142**:1165-76 and erratum *Am. J. Epidemiol.,* 1996;**144**:716). The earlier results were cited in the report of the IARC Working Group. Additional comments on this paper follow:

i) Where categorical results were presented in the recent publication, the categories used for analysis were quite different from those presented in the earlier work. The authors do not explain the reasons for changing the categorization scheme.

ii) The updated dose-response relationship did not reach the level of statistical significance previously reported by the authors and cited within the report of the IARC Working Group.

iii) Although Becher et al. indicated that analyses were conducted for both all cancer combined and lung cancer, only the results for all cancer combined were presented. It is unclear why the authors did not present the lung cancer results.

c) **Bertazzi et al., *Environ. Health Perspect.,* 1998;106(Suppl. 2):625-33.** This publication is the written version of the paper orally presented at the November 1996 meeting at the German Cancer Research Center. The cancer outcome data are

identical to those presented by Bertazzi et al. in the publication (*Epidemiol.*, 1997;8:646-52) cited in the Draft Background Document. The same reservations cited above, therefore, apply to this publication as well.

d) Flesch-Janys et al., *Environ. Health Perspect.*, 1998;106(Suppl. 2):655-62.
The cohort presented in this analysis is the same one presented in the paper by Flesch-Janys et al. (*Am. J. Epidemiol.*, 1995;**142**:1165-76) that was cited in the report of the IARC Working Group.

i) The model developed by the authors could explain only about one-quarter of the variability that was observed in serum levels of TCDD.

ii) The observed mortality from all causes in this cohort was higher than expected based upon mortality in the general population. This is a highly unusual finding for any working population, as workers tend to be healthier than the population at large (the healthy worker effect). This finding is in direct conflict with most other industrial cohorts of TCDD-exposed workers. It is unlikely that such an apparent generalized increase in mortality occurred because of a causal association with TCDD exposure and more likely represents some systematic error in effect estimation.

iii) No gradient of risk of lung cancer was observed using either TCDD or TEQ dose levels, arguing against a causal association.

iv) No gradient of risk of hematopoietic and lymphatic cancer was observed using either TCDD or TEQ dose levels, arguing against a causal association.

v) The risk gradient for all cancers combined with TEQ (that was cited in the report of the IARC Working Group as evidence of a dose-response relationship) was not observed in this analysis. As previously noted, dose-response analyses published by these authors on the same data set were the subject of a published erratum. In light of previous calculation errors and continuing apparent discrepancies in reported results, it is difficult to assign much weight to this evidence.

e) Hay et al., Ann. N. Y. Acad. Sci., 1997;837:138-59.

i) This is a study of phenoxy herbicide applicators. Although mentioned in the report of the IARC Working Group, studies of these types of populations were not given much weight. To quote the report directly: "In these studies, direct evidence of exposure to 2,3,7,8-TCDD is often lacking. The limited data available indicate that exposure to 2,3,7,8-TCDD is likely to be substantially lower than exposure in industrial cohorts" (p. 165). The present study did not have access to direct measurements of TCDD exposure, and there is no reason to believe that exposure was higher than other applicator populations.

ii) The study population was small (N =225), and thus estimates of outcomes were imprecise, as reflected by very broad confidence intervals.

iii) Overall mortality in this population of workers was 50% higher than would be expected based upon mortality in the general population. As stated for the preceding paper, the absence of a healthy worker effect across all causes of death is unlikely to arise from a causal association with a work-related exposure. More likely, this pattern suggests a systematic error in effect estimation.

iv) These workers were exposed to a variety of chemicals, including 2,4-D and 2,4,5-T, as well as polychlorinated biphenyls and polychlorinated dibenzofurans. It is impossible, therefore, to assign to 2,3,7,8-TCDD as the cause of any observed increase in overall or cancer mortality.

f) Kogevinas et al., *Am. J. Epidemiol.*, 1997;145:1061-75.

i) This paper was included in the report of the IARC Working Group as it had been accepted for publication and was in press at the time of the meeting. Data from this report were cited in Table 38 of the IARC report and thus were highlighted in the conclusions that were drawn at that time.

ii) The observed small excess of mortality from all cancers combined in this cohort did not correlate with duration of exposure, a proxy of dose. The absence of a gradient of risk with increasing dose argues against a causal interpretation. The authors were appropriately circumspect in their interpretation of this finding, stating that: "exposure to . . . TCDD and other chlorinated dioxins *may* (my emphasis) be associated with a small increase in overall cancer risk."

iii) The observed small excess of lung cancer mortality in this cohort also did not correlate with duration of exposure, and thus is unlikely to represent a causal association.

iv) The observed small excess of non-Hodgkin's lymphoma mortality in this cohort did not correlate with duration of exposure, and thus is unlikely to represent a causal association.

v) The observed modest excess of soft tissue sarcoma mortality is entirely attributable to the U.S. cohorts included in the NIOSH investigation (Fingerhut et al., *N. Engl. J. Med.*, 1991;324:212-18). Lack of confirmation in any of the other international cohorts and the absence of a monotonic dose-response relationship argues against a causal interpretation.

g) **Lynge, *Environ. Health Perspect.*, 1998;106(Suppl. 2):683-8.**

i) This cohort was included in the report of the IARC Working Group.

ii) No excess risk was observed for all types of cancers combined.

iii) No excess risk was observed for lung cancer.

iv) Small excesses in risk of soft tissue sarcoma and non-Hodgkin's lymphoma were observed, but these results were not statistically significant. The soft-tissue sarcomas occurred in workers with relatively short duration of exposure (two subjects were only three months in the plant), and thus, it is unlikely that these outcomes reflect an underlying causal relationship with exposure to TCDD.

h) **Michalek et al., *Am. J. Epidemiol.*, 1998;148:786-92.**

i) This report provides the mortality experience through 1993 of veterans of Operation Ranch Hand, the military unit responsible for aerial spraying of herbicides in Vietnam. These findings were reported previously (Ketchum and Akhtar, Interim Technical Report AL/AO-TR-1996-0068, 1996) and were included in the report of the IARC Working Group.

ii) There were fewer cancer deaths among exposed veterans than were expected.

iii) The subgroup of veterans with the highest measured serum dioxin levels (enlisted ground personnel) did not demonstrate any increased risk of cancer.

iv) A small excess of lung cancer deaths was observed among veterans followed at least 20 years, but chance could not be excluded as an explanation for this finding.

v) The sample size was too small to evaluate the risks of other specific types of cancer.

Tab 6

DATE

XXXX
XXXX
XXXX
XXXX

Dear Dr. _____:

I am writing to ask your assistance in evaluating the available scientific data relevant to a proposal to remove saccharin from the National Toxicology Program's Report on Carcinogens. One of the most significant responsibilities of the National Toxicology Program (NTP) is the preparation of the Report on Carcinogens. The Public Health Service Act of 1978 requires that the Secretary, Department of Health and Human Services (DHHS), shall submit this report to Congress. The responsibility for preparation of this report has been delegated by the Secretary to the NTP. The criteria by which substances are listed in the report were revised in 1996, taking advantage of recent advances in cancer biology and molecular toxicology, allowing the use of data from these and other "mechanistic" studies, and all other relevant information, to determine whether a substance should be listed in the report. A copy of the revised criteria are attached for your information as Tab A.

In 1997, 14 substances or exposure circumstances were considered for listing in or removal from the Ninth Report on Carcinogens. Formal consideration of a petition to delist saccharin, which is currently listed in the Report as *reasonably anticipated to be a human carcinogen* (based on results from animal studies), was conducted as part of this review. This included scientific review by an NIEHS staff review committee (RG1), an NTP Executive Committee Interagency working group (RG2) and an outside, scientific peer review by a Subcommittee of the NTP Board of Scientific Counselors (Report Subcommittee) which was held in an open public forum. In addition, public review and comment were solicited and received on the petition to delist saccharin. All available data relevant to the criteria for inclusion or removal of saccharin from the report were evaluated by the three scientific review committees. The votes of the three committees were not consistent and they forwarded different recommendations concerning the petition to delist saccharin. The RG1 (by a vote of 7 to 3) and the RG2 (by a vote of 6 to 2) recommended that saccharin should be delisted from the Report on Carcinogens. The Report Subcommittee (by a vote of 4 to 3) recommended that saccharin should remain listed in the Report on Carcinogens as *reasonably anticipated to be a human carcinogen*. The NTP also received public comments in favor of delisting as well as retaining the current listing in the Report.

The issues surrounding the review of saccharin for possible delisting from the Report are complex and difficult with scientific disagreements over the interpretation of the available data. The critical issues identified in the earlier scientific reviews include:

* The adequacy of existing epidemiology data, particularly as it relates to reported increased incidences of bladder tumor formation in certain small populations. Most of the epidemiology studies have examined associations between urinary bladder cancer and artificial sweeteners, rather than saccharin per se. None of the epidemiology studies identified an increased risk in the general population. Some epidemiology studies have identified an enhanced risk of developing urinary bladder cancer associated with exposure to artificial sweeteners in some case-control subgroups (involving small numbers).

* The levels of human exposure may be much closer to the no-observed-effect-levels for urinary bladder cancer in male rats than is generally assumed. Calculations based on body weight indicate a 100-fold difference between the no effect level in rats and the daily human consumption rate. The ratio is 50-fold for children. If the calculation is done based on surface area, there is a 20-fold difference for adults and a 16-fold difference for children.

* The mechanism of urinary bladder tumor formation in male rats as it relates to other test species (especially female rats and male and female mice) and to humans. The results of mechanistic studies have shown that certain physiological conditions must be simultaneously or sequentially present for induction of urinary bladder carcinogenesis in the rat. These include a urinary pH greater than 6.5, increased urinary sodium concentration, increased urine volume, decreased urine osmolality, presence of urinary crystals or precipitate, and damage to the urothelium resulting in a proliferative response. All these conditions have been studied extensively in male rats but less so in female rats and not in other test species.

* The adequacy of the data for tumor formation in laboratory animals at target sites other than the urinary bladder. The rat is the test species from which the majority of data related to the carcinogenicity of saccharin has been generated. Saccharin-induced carcinogenesis in rats shows a sex predilection for males, an organ specificity for urinary bladder and a dose response when approximate lifetime exposure to dietary concentrations of 1 to 7.5 percent of sodium saccharin begin early in life. The mouse is the only other test species where studies have been reported with positive carcinogenicity results following exposure to saccharin. Of the three "positive" mouse studies identified, two involved the surgical implantation of saccharin-containing cholesterol pellets into the urinary bladders and resulted in development of malignant urothelial neoplasms. In the third study, exposure to dietary sodium saccharin was reported to result in increased incidences of malignant thyroid neoplasms.

You are widely recognized as a leader in the area of chemical carcinogenesis and cancer biology. I am therefore soliciting your review and comments concerning the scientific issues identified above and any other matters you believe pertinent to this problem before I make my recommendation to the Secretary, DHHS, concerning the listing or delisting of saccharin in the Ninth Report on Carcinogens. I am enclosing for your information the final draft of the Background Document used for the review of saccharin; the Board Subcommittee's primary and secondary reviewers' written comments for saccharin; the overhead slides used by Dr. Robert Maronpot, NIEHS, in his presentation of the saccharin petition to the Board Subcommittee; the public comments received concerning saccharin; the minutes of the Board Subcommittee meeting; the most recent Federal Register Notice concerning the Ninth Report on Carcinogens which includes a request for final public comment on saccharin and the other nominations reviewed in 1997; and the Seventh Report on Carcinogens which is the most recent version of the Report and which contains the listing for saccharin on page 352. Please contact Dr. C.W. Jameson at (919) 541-4096 or by e-mail at jameson@niehs.nih.gov if you have any questions concerning the information provided or need assistance in obtaining additional references.

I would be most appreciative if you could provide your written comments concerning saccharin to me by May 15, 1998, the deadline for receipt of final public comment on saccharin. Your comments are important to me as I review all relevant information before making my recommendation to the Secretary concerning saccharin. I thank you in advance for helping to address this very important human health issue.

Sincerely,

Kenneth Olden, Ph.D.
Director

Enclosures:
Tab A - Current Criteria for Listing in the Report on Carcinogens
Tab B - Background Document for Saccharin
Tab C - NTP Subcommittee Reviewers' Written Comments
Tab D - Dr. Maronpot's Slides
Tab E - Public Comments received concerning the delisting of saccharin
Tab F - Draft minutes for the October 30 & 31, 1997, NTP Board Subcommittee meeting
Tab G - Federal Register Notice soliciting final public comment on 1997 Report on Carcinogens nomination reviews
Tab H - The Seventh Report on Carcinogens