DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR CHLORODIBENZOFURANS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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Comments provided by Peer Reviewer #1

COMMENT 1: The profile acknowledges the importance of the Toxic Equivalency (TEQ) approach to evaluating CDFs but doesn't fully explain what is presented in Haws et al. (2006) and Van den Berg et al. (2006). In Table 2 of Haws et al. (2006) the number of retained mammalian relative potency estimates (REPs) in the REP_{2004} Database are shown. There are a total of 213 CDF REPs with 2,3,4,7,8-Pentachlorodibenzofuran having 99.

RESPONSE: *It is beyond the scope of the CDFs toxicological profile to provide a detailed discussion of the development of the World Health Organization (WHO) TEFs. The following statement was added to Section 2.1:*

For additional information on the development of the TEFs, see Haws et al. (2006) and Van den Berg et al. (2006).

COMMENT 2: In Table 2-1 of the Toxicological Profile the summary "WHO 2005 Toxic Equivalency Factors (TEFs)" are listed. These TEFs are relative to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin which was assigned a TEF of 1. 2,3,4,7,8-Pentachloro-dibenzofuran was assigned a TEF of 0.3 which is a high value and is three times the TEF of 2,3,7,8-Tetrachlorodibenzofuran and three times the TEF of each of the four Hexachlorodibenzofurans. The other CDFs have TEFs of 0.03 or less.

RESPONSE: *The following footnote was added to Table 2-1:*

[a]TEFs are relative to the toxicity of 2,3,7,8-TCDD.

COMMENT 3: Everett (2020) investigated dioxin-like compounds in human blood in the 1999-2004 National Health and Nutrition Examination Survey (NHANES). At that time 2,3,7,8-Pentachloro-dibenzofuran was found to be above the maximum limit of detection in over 25% of the adult US population. Given the TEF of 0.3 and substantial amount in blood in the US population, the information in Figure 1-3, Figure 1-6, and Table 1-2 should be highlighted.

RESPONSE: *Figures 1-3 and 1-6 present information on sensitive targets of toxicity and dose-response data for 2,3,4,7,8-pentaCDF; Table 1-2 presents the Minimal Risk Level (MRL) values for this congener. This information is discussed in greater detail in Chapter 2. The intent of the toxicological profile is to present the toxicity data for all CDF congeners, rather than highlight one particular congener.*

COMMENT 4: The cut-off values reported for Everett and Thompson (2014, 2016) were not based on a per lipid basis, but rather on a whole blood basis. The numbers should be ≥51.74 fg/g whole blood in Everett and Thompson (2014) and ≥54.02 fg/g whole blood in Everett and Thompson (2016). Also, the sample in Everett and Thompson (2016) should be "teens and young adults, 12-30 years old."

RESPONSE: *The units were corrected for the Everett and Thompson (2014, 2016) studies:*

Section 2.10

In a study of teens (ages 12–19 years) participating in the National Health and Nutrition Examination Survey (NHANES), no association was found between the incidence of nephropathy and blood 2,3,4,7,8-pentaCDF levels ≥54.02 fg/g whole blood (Everett and Thompson 2016).

Section 2.13

A study of NHANES participants with blood 2,3,4,7,8-pentaCDF levels ≥51.74 fg/g whole blood found an increased risk of diabetes with or without nephropathy (Everett and Thompson 2014).

COMMENT 5: Releases of CDFs from chlorine bleaching in pulp and paper mills in the US ended a long time ago. The authors can acknowledge this process as a potential source but should also note it is no longer a problem.

RESPONSE: *A statement was added to Section 5.1 that chlorine bleaching of pulp or paper is no longer a relevant source of CDFs in the United States. The discussion of the release of CDFs from chlorine bleaching in pulp and paper mills in Section 5.3.2 has been revised:*

Historically, an important source of CDFs in surface water is the discharge of effluents from pulp and paper mills that use the bleached kraft process. The concentrations of 2,3,7,8-tetraCDF in the treated effluents from five bleached kraft pulp and paper mills in the United States ranged from not detected (0.007 ppt) to 2.2 ppt with a mean value of 0.54 ppt, but the waste water sludges contained 2,3,7,8-tetraCDF at a mean concentration of 0.37 ppb (Amendola et al. 1989). The effluent from a kraft pulp mill from Jackfish Bay, Lake Superior, contained tetraCDFs in concentrations ranging from 0.3 to 1.3 ng/L (9.3–1.3 ppt) (Sherman et al. 1990). Due to guidelines under the Clean Water Act, this is no longer a source of CDF releases in the United States.

COMMENT 6: Table 5-1 appears to be missing a word in the title. I think it should read: Facilities that Produce, Process, or *Use* Dioxin-like Compounds.

RESPONSE: *The title for Table 5-1 was corrected.*

COMMENT 7: Table 5-4 has problems with the column headings. "Tetra" appears twice and "Hepta" does not appear at all. Please check this table closely.

RESPONSE: *The column headings have been corrected in Table 5-4.*

Comments provided by Peer Reviewer #2

COMMENT 1: The profile is a very broad number toxicological topics and studies reviewing a significant amount data from the almost 600 publications cited in the profile. This is very challenging as a significant amount of these studies and resulting data were produced under many different conditions making it difficult to come up with meaningful relationships. The profile summarizes and organizes large amounts of important data pertaining to CDFs.

RESPONSE: *No response needed.*

COMMENT 2: Chapter one is an excellent summary of the exposure of a wide variety animals to chlorinated dibenzofurans and how it is related to Public Health. The profile lists a number of instances for which health effects occur in humans as a result of accidental or occupational exposure. The focus is on the main CDF contamination episodes that have occurred at Yusho and Yu-Cheng. These are significant historical events that have enabled a large number of studies carried out identifying a wide variety of endpoints. These are covered in great detail in the document. A number of other exposure instances including proximity to contaminated sites, worker industrial exposure and exposures such as cigarette smoking are discussed. The exposures have lead to a series of health effects that are covered in detail. The main challenge in human exposures is that it is very difficult to identify the actual dose because toxicological studies for compounds like halogenated aromatic compounds are not carried out directly on humans and humans are not typically exposed a single toxicant. This can make it very challenging to determine what a specific endpoint is as a result of a specific toxicant. CDFs have a specific common mode of action through the AH receptor and allows a TEF approach to be used which is mentioned a number of times in the document.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

COMMENT 3: There are a significant number of dosing studies and effects listed for animals focusing mainly on mammals including mice, rats, Guinea pigs and monkeys. Mammals especially monkeys are the closest group of animal species to humans which are also included in the mammal group, and these effects and endpoints can be extrapolated to humans. Effects only observed in animals are reported likely because there is no data for human exposure available as humans are not directly exposed to toxic substances and/or the levels dosed in animal species were below the NOEAL for human exposure.

RESPONSE: *No response needed.*

COMMENT 4: For the most part, exposure conditions have been adequately defined. Species, dosing concentrations and mode of application including oral, dermal, inhalation and subcutaneous injection of specific CDFs including duration listed as acute, intermediate and chronic and/or exact dosing schedule and times are detailed. Because there are large amounts of exposure studies listed in the document and it is difficult to provide extensive details for each study.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

COMMENT 5: Unfortunately, there is not sufficient data from the studies referenced to calculate minimal risk levels (MRLs) for all of the 2,3,7,8-substitited CDFs. MRLs were only calculated for

1,2,3,7,8 penta CDF, 2,3,4,7,8 penta CDF and 1,2,3,6,7,8 hexa CDF. MRLs were estimated for the 2,3,7,8-substitued CDF congeners that did not have sufficient data using a TEF approach as well as three CDFs listed above. The TEF calculated CDFs are in good agreement with the MRLs that were calculated using first principles from dosing and exposure studies. Uncertainty and modifying factors range from two to three orders of magnitude. Uncertainty factors and other safety factors are routinely used and can be as high as five orders of magnitude. Three orders of magnitude is a reasonable level of safety considering that humans typically are not as sensitive as other animals/mammals to toxic substances. Although data is lacking in a significant number of areas, the TEF approach, potentially with larger uncertainty factors could be used to increase the number of MRLs. They could be identified as tentative MRLs.

RESPONSE: *TEF-derived MRLs for the 10 CDF congeners with toxicity data are presented in Table A-3.*

COMMENT 6: Chapter 2 provides a detailed summary of health effects in animals and humans in relation to the various exposures and resulting outcomes. Seventeen different endpoints are discussed. Conclusions are made for the majority of the endpoints. Due to the extremely large amount of data cited in the document from the numerous studies it is difficult to report detailed conclusions for all of the endpoints.

RESPONSE: *No response needed.*

COMMENT 7: A number of human designed studies were included in the text focusing mainly on the Yusho and Yu-Cheng exposures. Other exposures including Agent Orange contaminated sites in Vietnam and other contaminated sites were reported. The Yusho and Yu-Cheng exposures studies reported a number of results that occurred years after these incidents ensuring a sufficiently long period of exposure to account for observed health effects. There is a significant challenge in controlling for confounding factors in many of these studies as there are many confounding factors due to the exposure from multiple various chemicals and other stressors. The Yusho and Yu-Cheng studies included large populations which enabled control for some of the confounding factors to allow some conclusions to be made. Due to the large number of studies and the large variation in the various studies, it was challenging to come up with final conclusions. Limitations for the studies were reported for some of the studies. Unfortunately in many cases it is not possible to account for or address the limitations.

RESPONSE: *ATSDR agrees with the Reviewer that the interpretation of many of the epidemiological studies is limited by the lack of control for potential confounders.*

COMMENT 8: A significantly large number of designed animal studies were reported in the text. Some of the studies had an adequate number of animals in the test group and others specifically those involving monkeys often had a small number, likely as a result of difficulties in obtaining a large number of animals for the study. In such cases it makes it difficult to use a large amount of different doses and/or exposure durations. The low number of animals in the monkey studies does not negate the study as the information is useful even though the statistical power is low. One of the major challenges is the variable response between animal species. In order to have a significant amount of data in order to obtain useful conclusions regarding a sufficient number of dosed groups and sufficient magnitude in dosing levels a larger number participants is typically needed. This was the case for many of the mice and rat studies.

RESPONSE: *ATSDR agrees with the Reviewer that many of the studies in species other than rats and mice were limited by the small number of animals per group or small number of doses tested.*

COMMENT 9: A variety of animals including mainly mammals, mice, rats, Guinea pigs and monkeys were reported. For the CDFs for which MRLs have been determined, data from rat studies were used. Rats are readily available for toxicity studies and there generally is a significant amount of data available for rat based dosing studies for the majority of toxic compounds making them a preferred species to study as it enables comparison between the different toxicants. Genetically, monkeys are most closely related to humans and could be considered the most appropriate species, but they can be more sensitive in some endpoints than rats.

RESPONSE: *The monkey studies were considered in the MRL derivations; however, these studies either identified higher lowest-observed-adverse-effect level (LOAEL) values or were not considered an adequate (due to small numbers of animals tested) basis for an MRL.*

COMMENT 10: Data for dose response is reported for a large number of studies for the majority of CDF congeners for animal studies. The challenge for humans is determining the actual dose of the compound being evaluated. In many cases, for example Agent Orange exposure in Vietnam, there are other potential interfering compound like 2,3,7,8-TCDD which makes it difficult to identify the exact link between dose and response.

RESPONSE: *ATSDR agrees that the available epidemiological data have limited usefulness in establishing dose-response relationships due to co-exposure to a number CDDs, CDFs, and dioxin-like polychlorinated biphenyls (PCBs), which have similar mechanisms of action.*

COMMENT 11: The report cites 579 references and includes many of the relevant references in the field focusing on original studies for toxicity and determination of MRLs. Unfortunately, many of these studies have administered different doses, conditions and endpoints resulting in challenges when comparing the studies.

RESPONSE: *No response needed.*

COMMENT 12: NOEALs and LOEALs are reported for a large number of studies and compounds in the tables and figures. Splitting exposures into less serious and serious LOEAL levels provides a greater number of categories for assessing dose/exposure effects. This is important especially for the less serious LOEAL which can aid in the development of MRLs.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

COMMENT 13: The main mechanism of action for CDFs – binding to the Ah receptor is indicated in the text as well as for a number of other mechanisms including structure toxicity relationships and oxidative stress.

RESPONSE: *No response needed.*

COMMENT 14: Chapter 3 summarizes the toxicokinetics, susceptible populations, biomarkers and chemical interactions. There is significant discussion regarding absorption, distribution and excretion. The main conclusion is that CDFs are eliminated mainly in feces and can also be eliminated in urine. Adsorption occurs mainly through oral ingestion. Rates of absorption were as high as 100%. A number of studies including ^{14}C studies subcutaneous injections were carried out. Data showed that ^{14}C- 2,3,7,8-CDF was quickly distributed to lipid containing organs.

RESPONSE: *No response needed.*

COMMENT 15: The report also provides information on a number of pharmacokinetic and pharmacodynamic models. The section is brief and can use more information.

RESPONSE: *As per the toxicological profile guidance document, physiologically based pharmacokinetic (PBPK) models that are not used to derive MRLs are briefly discussed in Section 3.15.*

COMMENT 16: There is significant discussion on toxicokinetics for both humans and animals. More details can be provided for animal toxicokinetics and how it can relate to humans.

RESPONSE: *Studies providing additional information on toxicokinetic differences between humans and experimental animals were not identified for CDFs. The Reviewer did not provide supportive references.*

COMMENT 17: Effects on children are reported for a limited number of studies. More information would help, but unfortunately little information is available. There also is little information on unusually susceptible populations.

RESPONSE: *ATSDR agrees with the Reviewer that there are limited data on children's susceptibility; the need for additional studies is discussed in Section 6.2 of the profile.*

COMMENT 18: Biomarker of exposure and effect are discussed in significant detail. One of the challenges is connecting all the biomarkers, of which there can be a very large number, to show that there is a relationship to dose, response and endpoints can make it difficult in correlating the many studies reported.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

COMMENT 19: There also is discussion on the interactive effects between CDFs as well as CDFs and CDDs. Most of the data indicate that dioxin-like compounds are additive using at TEF scheme. Some of the data reported indicate that results can be antagonistic, which can be beneficial if the chemicals are less toxic than the CDFs and harmful if they are more toxic. This is described in significant detail in the document.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

COMMENT 20: Chapter 4 provides the chemical and physical property information. There is a significant amount of information provided. Unfortunately, data is not available for a number of

parameters. Most of these studies have not been carried out or are not published in the scientific literature.

RESPONSE: *No response needed.*

COMMENT 21: Chapter 5 discusses the potential for human exposure. There is very little information available on the production, import/export, use and disposal of CDFs because they were never commercially produced at any significant levels other than for chemical standards. The major source of exposure is in food and levels are typically low. Higher levels are present in waste and specifically hazardous waste.

RESPONSE: *No response needed.*

COMMENT 22: The point of release transport to the receptor population is reported for a number of the CDFs. The text reports information regarding levels at NPL sites where available. Data is reported for some CDFs for transport, partitioning and degradation.

RESPONSE: *No response needed.*

COMMENT 23: There is a significant amount of data reported for levels in the environment including air, land/soil, water, fish and other foods. Results are reported for workers at chemical plants and other chemical related industries which were exposed to higher levels. There were also higher exposures to infants that were breast feeding resulting increased body burdens.

RESPONSE: *No response needed.*

COMMENT 24: Chapter 6 reviews the adequacy of the database. There are data gaps listed for most of the categories. These are outlined in detail in the text. The majority of data is for oral dosing studies with a small number reported for dermal exposure. Additional data as outlined in the report is needed. All the data is presented in a neutral non-judgemental manner.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

COMMENT 25: Chapter 7 summarizes the international and national regulations and advisories and guidelines with respect to CDFs in air, water and drinking water and food. There are very few guidelines for furans alone. The majority of the guidelines are for combined CDD and CDF TEQ values.

RESPONSE: *No response needed.*

COMMENT 26: Appendix A summarizes all of the Minimal Risk Level worksheets for the chlorinated dibenzofurans. Due to the lack of data from human or animal studies, MRLs are not reported for many of the CDF congeners for oral exposure. No MRLs are reported for inhalation studies also due to a lack of sufficient data. This is a significant challenge and one area where data gaps are large.

RESPONSE: *ATSDR agrees with the Reviewer that the lack of adequate acute-, intermediate-, and chronic-duration oral studies for most CDF congeners is a data gap and the need for additional studies has been identified as a data need in Section 6.2 of the profile.*

Comments Provided by Peer Reviewer #3

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 1: The human data reported in the document is from two different events that occurred a number of decades ago, one being in Yusho, Japan and the other in Yu-Cheng, Taiwan. There is good reason to believe that the health effects detected in both of these scenarios were due to the chlorodibenzofurans (CDFs), but the case is somewhat complicated by the fact that in both circumstances there were other categories of molecules to which the individuals were also exposed. Although it is doubtful that these other molecules were major players in the health effects suffered by the humans in these instances, it is possible that these other molecules function to potentiate the difficulties associated with the CDFs. Since these various molecules have the potential to function as agonists of the aryl hydrocarbon receptor (AhR), although at much lower potencies than the CDFs, it seems likely that these other molecules function to essentially enhance the dose of the CDF by a small percentage.

RESPONSE: *ATSDR agrees with the Reviewer; the possible contribution of other chemicals to the observed toxicity is discussed in several places in the profile, particularly Section 2.1.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 2: As time passes the various biological outcomes connected with these chemical agents will become more sophisticated. In many circumstances these outcomes will be detected in well controlled animal experiments because it is hopefully unlikely that we will be obtaining significant amounts of human data. Furthermore, as the background concentrations of these compounds in the environment and in human bodies becomes lower, the ability to detect consequences that can be attributed to the compounds becomes more prevalent. Thus, the information that has been gathered from the Yusho and Yu-Cheng was only gross human outcomes that were easily attributable to these two incidents. If these compounds contribute to more common chronic diseases such as cardiovascular disease, diabetes, immunosuppression or neurodegenerative diseases, it may require years to determine the connections and they are unlikely to be determined in humans (although *in vitro* techniques using human cells may be useful for this purpose).

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 3: The exposure conditions have been adequately described. However, it seems that mono-, di-, and trichlorinated CDFs are not considered according to statement in the second paragraph of chapter 2 (lines 586-587). If this is a universal omission for the entire document, then it should be mentioned in Chapter 1.

RESPONSE: *The following text was moved from Section 2.1 to the introduction in Chapter 1:*
Chlorodibenzofurans (CDFs) are a class of structurally similar chlorinated hydrocarbons containing two benzene rings fused to a central furan ring (see chemical structure in Section 4.1). Based on the number of chlorine substituents (one to eight) on the benzene rings, there are eight homologues of CDFs (monochlorinated through octachlorinated). Each homologous group contains one or more isomers. There are 135 possible CDF isomers, including 4 monochlorinated dibenzofurans (monoCDFs), 16 dichlorinated dibenzofurans (diCDFs), 28 trichlorinated dibenzofurans (triCDFs), 38 tetrachlorinated dibenzofurans (tetraCDFs), 28 pentachlorinated dibenzofurans (pentaCDFs), 16 hexachlorinated dibenzofurans (hexaCDFs), 4 heptachlorinated dibenzofurans (heptaCDFs), and 1 octachlorinated dibenzofuran (octaCDF). The term congener is used to refer to any one particular isomer. Mono-, di-, and trichlorinated CDFs are not considered in this profile.

Minimal Risk Levels

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 4: In the case of inhalation exposures, there has been no MRLs derived because there were no studies performed. It is my opinion that this is the most prudent approach because there is no data. I would anticipate that much like the dioxins that bioavailability of CDFs by the inhalation route, much like the gastrointestinal route, would be almost complete (F ~ 0.9-1.0) and that dermal bioavailability would be approximately one half of the inhalation/gastrointestinal values (F ~ 0.4-0.5). Earlier in this section it was stated that there were only two studies identified where dermal exposures were used but the MRL section does not state any MRL for dermal exposure studies. It is assumed that like inhalation studies that it was decided that there was insufficient data to derive an MRL, but it seems that like inhalation, it should be stated that this was the case.

RESPONSE: *As noted in the introduction in Appendix A, ATSDR does not derive MRLs for the dermal route.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 5: In general, uncertainty factors are not scientifically derived as indicated by the fact that they are either numerically a factor of 3 or 10 irrespective of the variable. However, they serve a public health purpose by applying the concept of the precautionary principle to the regulations of chemicals. There may be circumstances where the uncertainty factors are not totally protective, but the general application of uncertainty factors will protect against the vast majority of public health issues. The only way to delineate those circumstances where the uncertainty factors are not protective is to understand at a mechanistic level how chemicals interact with biological systems and the peculiarities of the biological systems.

RESPONSE: *If data are available, ATSDR derives chemical-specific uncertainty factors. The available toxicokinetic data were not considered adequate for deriving chemical-specific uncertainty factors for CDFs. Thus, the Agency used its default uncertainty factor of 10 or 3.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 6: The MRL database assessment appears to be appropriately assembled.

RESPONSE: *No response needed.*

Chapter 2

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 7: The results for each type of health effect are summarized at the end of each specific section which are organized by various pathological outcomes and there is an overall summary in the introduction to this section. There is not major disagreement with the majority of these summaries. However, it is proposed that the mechanism section is lacking regarding biological functions of the AhR that are nongenomic (see question 10 in this section) and there is some concern about summarizing the carcinogenesis of these compounds as group 3, when the only long-term bioassay of one of the congeners has been positive, and TCDD, which is presumed to have the same toxicodynamics as the CDFs, is a carcinogen.

RESPONSE: *A discussion of nongenomic mechanisms involving the AhR receptor was added to Section 3.4:*

> Studies with 2,3,7,8-TCDD provide *in vitro* evidence of a nongenomic mechanisms involving the Ah receptor, but not requiring ARNT (Matsumura 2009). These mechanisms appear to contribute to the inflammatory response via cytosolic phospholipase A2 (cPLA2), Cox-2, Src kinase, and other protein kinases and phosphatases.

In 2012, IARC reconsidered the cancer classification for 2,3,4,7,8-pentaCDF and categorized it as carcinogenic to humans (group 1). The text in Sections 1.2 and 2.19 has been revised to reflect this change.

Section 1.2:

> The International Agency for Research on Cancer (IARC 2012) concluded that 2,3,4,7,8-pentaCDF is carcinogenic to humans; the agency also concluded that other CDF congeners are not classifiable as to their carcinogenicity to humans (Group 3) (IARC 1997).

Section 2.19:

> IARC (2012) concluded that 2,3,4,7,8-pentaCDF is carcinogenic to humans (Group 1). Other CDF congeners are not classifiable as to their carcinogenicity to humans (Group 3) (IARC 1997).

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 8: It is likely that the CDFs have the same wide variety of health outcomes as the chlorodibenzodioxins (CDD). According to Linda S. Birnbaum (Curr Opin Toxicol. 2017 Feb; 2: 120–

123.): "Dioxins cause a wide range of effects, ranging from molecular to organismal. Effects are context dependent but can occur in almost every tissue during some life stage and in both sexes of animals. Biochemical effects include induction of Phase I, II, and III metabolizing enzymes and transporters, genes involved in proliferation, cytokines, growth factors and receptors, and more. Clearly adverse effects range from lethality, wasting, and gonadal and lymphoid atrophy, at high exposures, to hyperplasia, metaplasia, carcinogenesis, endocrine disruption, reproductive and developmental toxicity, including functional developmental toxicity, dermal toxicity, immunotoxicity, neurotoxicity, hepatic toxicity, cardiovascular toxicity, bone and teeth toxicity, cardiovascular toxicity, and diabetes [1]. Of greatest concern are the developmental alterations associated with prenatal exposure occurring at the high end of the background population: decreased learning and memory, altered behaviors, suppressed immune system, compromised endocrine systems, and impacts on growth [5]."

From a mechanistic perspective both CDDs and CDFs function as agonists for the Ah receptor and consequently it could be hypothesized that the same wide variety of biological outcomes would be expected from the CDFs as has already been determined for CDDs if administered at the appropriate dose and at the appropriate time.

RESPONSE: *ATSDR agrees and notes that the similarity of the mechanisms of action of CDFs with CDDs and dioxin-like PCBs is discussed in numerous places in the profile, particularly in Section 2.20.*

COMMENT 9: One issue that would be worth addressing regarding the human studies relates to the TEF values that are found in Table 2-1. There are essentially three categories of chemical compounds that have been detected in the exposures in Yusho and in Yu-Cheng, namely PCBs, CDFs and PCQs (polychlorinated quaterphenyls). Table 2-1 lists the quantitative TEF values for a number of CDDs, CDFs and PCBs. However, there are no values for the PCQs. Do these chemicals bind to the Ah receptor? If they do not, then it should be so stated. If they do bind to the receptor, then the TEF values for representatives of this class of molecules should be listed in Table 2-1. Even if the TEF values are relatively low compared to the CDFs, they may still contribute significantly to the hazard because the cumulative concentrations of these compounds were more than two orders of magnitude higher than the CDFs in the human exposure scenarios.

RESPONSE: *The TEFs listed in Table 2-1 are for dioxin and dioxin-like compounds; WHO did not include polychlorinated quaterphenyls in the TEF concept. According to Van den Berg et al. (2006), they have not been identified for possible future inclusion in this TEF concept. It is not known whether polychlorinated quarterphenyls act via the Ah receptor.*

COMMENT 10: One potential confounding variable for the results enumerated in Table 2-4 is that the subjects in the Chang et al. (2016) study were inhabitants of a location that presumably had high levels of dioxins. The results reported in the study found that abdominal obesity was associated with several tetra-, penta- and hexaCDF congeners in men, and some heptaCDF congeners in women. However, because of the potency of 2,3,7,8-TCDD for the Ah receptor, it may be that the obesity monitored in this study was a result of exposure to this compound. This inference is similar to the logic that was used to suggest that in Yusho and Yu-Cheng, it was CDFs that were the most potent compounds around and therefore the health effects were in fact due to these compounds.

RESPONSE: *ATSDR agrees with the Reviewer that interpretation of the results of the epidemiological studies is problematic due to co-exposure to other dioxin-like compounds, which have a similar mechanism of action as CDFs. This issue is discussed in Section 2.1 of the profile.*

COMMENT 11: In Tables 2.7, 2.8, and 2.9 where the biomarkers listed are chlorinated dibenzofurans, the studies were conducted in areas that were suggested to be high in background level of TCDD. This presumably represents a confounding variable for these studies. Is this stated somewhere in the test for these outcomes?

RESPONSE: *As noted in the Response to Comment 10, the issue of co-exposure to other dioxin-like compounds is discussed in Section 2.1. Co-exposure to other dioxin-like compounds, particularly 2,3,7,8-TCDD, is also discussed in other sections of the profile. For example, it is noted in Section 2.17 that studies of children living in areas of Vietnam that were sprayed with Agent Orange were likely exposed to high levels of CDDs, particularly 2,3,7,8-TCDD.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 12: It is not clear that a sufficient number of the congeners have been assayed (only one has been assayed) in an appropriate chronic exposure study for carcinogenesis. The only compound with a sufficient assay of carcinogenic potential is 2,3,4,7,8-pentaCDF, and it is suggested that the results from that assay were positive in that there were several types of cancer that were found to be induced by the exposure. According to Table 2-2, the cancer bioassay was performed in Sprague-Dawley rats and used only female rats, thus it would be beneficial to know the results of a cancer bioassay in male animals.

There are studies that have been performed that have indicated that three of the congeners (2,3,7,8-tetraCDF, 2,3,4,7,8-penta CDF, and 1,2,3,4,7,8-hexaCDF) were tumor promoters. In each of these instances the dermal administration of the CDF congener followed the administration of a cancer initiator, MNNG, and demonstrated over a 20 week period that the CDFs could perform as tumor promoters.

RESPONSE: *The National Toxicology Program (NTP 2006) cancer bioassay of 2,3,4,7,8-pentaCDF was only conducted in female rats. As noted by the Reviewer, there are limited data on the carcinogenicity of CDF congeners. The following text was added to Section 6.2:*

> ***Cancer.*** There are limited data on the carcinogenicity of CDF congeners in animals. The oral carcinogenicity database is limited to a chronic-duration study of 2,3,4,7,8-pentaCDF in female rats (NTP 2006). The remainder of the database consists of dermal tumor promotion studies on 2,3,7,8-tetraCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF. Additional carcinogenicity studies are needed to assess the carcinogenic potential of CDFs.

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 13: It is somewhat difficult to identify the most significant toxicological endpoint in the study. The toxicological profile indicates that the individual congeners basically have biological endpoints that are consistent with the idea that they are agonists for the Ah receptor and thus function to cause the wide variety of toxic endpoints that are known to be induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. This compound, as previously mentioned, induces a wide variety of different pathologies.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 14: Whereas the animal studies have, in general, appropriate doses used to evaluate a variety of different biological endpoints, the human studies have difficulties with respect to determining the dosage that people were exposed to as well as when they received the dose. This is because the studies analyzing the Yusho and Yu-Cheng cases were primarily retrospective studies trying to determine historical exposures.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 15: Not familiar with additional endpoints specifically by the CDFs.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 16: I am unaware of additional studies for deriving MRLs.

RESPONSE: *No response needed.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 17: NOAELs/LOAELs and in some cases benchmark doses have all been identified in order to estimate the thresholds of toxicological effects. These endpoints have not been determined for all studies because they are not appropriate parameters for determining mechanisms or other types of academic exercises in toxicology.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 18: I find these categories to be somewhat difficult to delineate. For example, among the various types of birth defects that might be induced which ones would be categorized as “serious” and “less serious”. Within developmental toxicology or teratology there is a similar delineation between malformations and variations.

RESPONSE: *ATSDR acknowledges that there is a considerable amount of judgement in determining whether an adverse health effect is a less serious LOAEL or a serious LOAEL. Serious effects are those that evoke failure in a biological system and can lead to morbidity or mortality. Examples of serious developmental effects include decreases in survival, marked decreases in body weight, some skeletal anomalies such as spina bifida or cleft palate, and visceral effects such as cardiac defects.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 19: Although the classic activity of ligands of the Ah receptor are through genomic transcriptional regulation, there are studies that indicate that the receptor activates nongenomic signal transduction pathways (Matsumura F. Biochem. Pharmacol. 77: 608-626, 2009). These pathways include activation of src-family kinases, intracellular calcium signaling as well as other inflammatory signaling. Furthermore, nuclear signaling via the Ah receptor may involve other transcription factors than the AhR-Arnt dimer, such as estrogen receptors. It would be scientifically valuable to know how many of the mechanistic actions of 2,3,7,8-TCDD are shared with the CDFs.

RESPONSE: *A discussion of nongenomic mechanisms was added to Section 3.4:*

> Studies with 2,3,7,8-TCDD provide *in vitro* evidence of a nongenomic mechanisms involving the Ah receptor, but not requiring ARNT (Matsumura 2009). These mechanisms appear to contribute to the inflammatory response via cytosolic phospholipase A2 (cPLA2), Cox-2, Src kinase, and other protein kinases and phosphatases.

COMMENT 20: It has recently been shown that CDFs have the potential to perturb epigenetic parameters early in life which may be critical in programming or imprinting the organism for eventual susceptibility to health problems in adult organisms or even to subsequent generations. This type of alteration has been referred to as developmental origins of health and disease (DOHD) and provides numerous challenges attempting to decipher the connections.

> Reference: Su KY, Li MC, Lee MW, Ho BC, Cheng CL, Chuang YC, Yu SL, Guo YL. Perinatal polychlorinated biphenyls and polychlorinated dibenzofurans exposure are associated with DNA methylation changes lasting to early adulthood: Findings from Yucheng second generation. Environ. Res. 170: 481-486 (2019 March).

RESPONSE: *ATSDR reviewed the Su et al. (2019) paper examining DNA methylation in male offspring of Yu-Cheng mothers. The results were considered preliminary and were not added to the profile. Su et al. (2019) concluded that "further studies were warranted to examine whether the observed [DNA] methylation changes alter gene expression."*

COMMENT 21: On page 71: "A likely mechanism involves the catabolism of T4 via T4 glucuronidation." If the precursor to T3 is removed via biotransformation (T4), then how does T3 increase?

RESPONSE: *A proposed mechanism for the increase in T3 levels has not been identified.*

COMMENT 22: A recent pathway by which these chemical compounds may impact the health of exposed humans is via the microbiome. Reference: Neamah WH, Busbee PB, Alghetaa H, Abdulla OA, Nagarkatti M, Nagarkatti P. AhR activation leads to alterations in the gut microbiome with consequent effect on induction of myeloid derived suppressor cells in a CXCR2-dependent manner. Int J Mol Sci 21(24): E9613 (2020 Dec 17). doi: 10.3390/ijms21249613.PMID: 33348596.

RESPONSE: *The Neamah et al. (2020) paper was added to the discussion of immunotoxicity mechanisms of action in Section 2.14:*

A study with 2,3,7,8-TCDD in mice found alterations in the gut microbiome which resulted in increasing the production of myeloid derived-suppressor cells (MDSCs) via Ah receptor activation (Neamah et al. 2020). The MDSCs have been shown to be immunosuppressive.

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 23: As an overall generalization, the conclusions are appropriate.

RESPONSE: *ATSDR thanks for the Reviewer for the comment.*

Chapter 3

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 24: There is a discussion of absorption, distribution, metabolism and excretion that summarizes a number of studies. However, the discussion omits some of the classical characteristic parameters for these processes. There is no mention of plasma protein binding of the CDFs. Although these compounds appear to bind to CYP1A2 in hepatic cells, it is not clear if they are bound when they are in the blood. Another parameter that would be valuable to the assessment is whether CYP1A2 is induced by the CDFs (and whether this gene contains a dioxin response element in the enhancer region). Is it appropriate to assume that when CDFs serve as a ligand for the Ah receptor, that the receptor binds to Arnt and then binds to the same dioxin response element as when the receptor is activated by 2,3,7,8-TCDD.

RESPONSE: *ATSDR did not identify information on plasma protein binding of CDFs. In Section 3.1.2, it is noted that CDF induces CYP12A.*

COMMENT 25: The toxicokinetic section of the report does not mention the volume of distribution. It could be hypothesized that this parameter would be relatively large based on the characteristics of the CDFs. There is mention of some of the metabolites that are produced in various species and this characteristic may differentiate the CDFs from the CDDs. However, the discussion of the biotransformation products is limited. It is suggested that CDFs undergo phase 1 biotransformations to both dechlorinate and to hydroxylate the ring structures as a result of Cyp1a1 activity (but not Cyp1a2 activity). But there is no quantitative assessment of what percentage of the parent compound is metabolized to these phase 1 products. Also, there is limited discussion of the production of phase 2 metabolites (there is a sentence that mentions both glucuronides and sulfates being produced). There is no mention of transport processes (phase 3) by either ABC transporters or SLC transporters. This may be

important because it is stated in the report that it is unknown how these molecules are transported across membranes.

A useful reference that demonstrates many of the concepts in this section with a non-human primate is as follows: Neubert D, Wiesmüller, Abraham K, Krowke R, Hagenmaier H. Persistence of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in hepatic and adipose tissue of marmoset monkeys. Arch. Toxicol. 64(6): 431-442 (1990) doi: 10.1007/BF01977624. PMID: 2125825. This paper shows in primates that half-lives for PCDFs were different for the various 2,3,7,8-substituted congeners than for the other forms, half-lives increased for congeners with increasing degrees of chlorination, and half-lives were dependent on when they were assessed because of the redistribution phenomenon.

RESPONSE: *No information on the volume of distribution or transport processes was located for CDFs. The Neubert et al. (1990) study was added to Section 3.1.2:*

> In marmoset monkeys subcutaneously administered a mixture of CDDs and CDFs, elimination half-times from adipose tissue and hepatic tissue increased with the degree of chlorination (Neubert et al. 1990). The location of the chlorines also influenced the elimination half-times with 2,3,7,8-substituted congers having longer half-times.

COMMENT 26: One other classical pharmacokinetic parameter that is not discussed is the clearance values. These values may be derived from the first-order elimination rate constants in the PBPK models.

RESPONSE: *Clearance values for CDFs were not located.*

COMMENT 27: Persistence of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in hepatic and adipose tissue of marmoset monkeys. Arch. Toxicol. 64(6): 431-442 (1990) doi: 10.1007/BF01977624. PMID: 2125825. This paper shows in primates that half-lives for PCDFs were different for the various 2,3,7,8-substituted congeners than for the other forms, half-lives increased for congeners with increasing degrees of chlorination, and half-lives were dependent on when they were assessed because of the redistribution phenomenon.

RESPONSE: *As noted in the Response to Comment 25, the Neubert et al. (1990) paper was added to Section 3.1.2. Additionally, a table of hepatic tissue and adipose tissue half-times for various CDFs was added to the profile.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 28: There is an appropriate discussion of the pharmacokinetic models. It is unknown if there are any appropriate pharmacodynamics models available.

RESPONSE: *ATSDR thanks the Reviewer for the comments.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 29: There is a discussion of the animal to human extrapolation for TCDD. It is presumed that this relationship was described because there was insufficient data to make that same comparisons between species for the CDFs. It is stated that the binding affinity of TCDD for the AhR in animals is approximately and order of magnitude higher in the laboratory animals than it is in the human. What is not mentioned is the number of dioxin response elements in the enhancer regions of specific genes which also differs between humans and laboratory animals.

A study that examined human immunological parameters and correlations with PCDDs/PCDFs was performed by a group that had previously examined non-human primates. Their overall assessment was as follows: "Adult humans certainly are less susceptible to this action of PCDDs/PCDFs than adolescent Callithrix jacchus." References: (1) Neubert R, Maskow L, Webb J, Jacob-Müller U, Nogueira AC, Delgado I, Helge H, Neubert D. Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system. 1. Blood cell receptors in volunteers with moderately increased body burdens. Life Sci. 66(22): 2123-2142 (2000). doi: 10.1016/0024-3205(93)90021-t.PMID: 8255162. (2) Neubert R, Golor G, Stahlmann R, Helge H, Neubert D. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 4. Effects of multiple-dose treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on peripheral lymphocyte subpopulations of a non-human primate (Callithrix jacchus). Arch. Toxicol. 66(4): 250-259 (1992); doi: 10.1007/BF02307170.PMID: 1514923

RESPONSE: *The Neubert et al. (1992, 2000) studies were not added to the profile because they discussed comparisons of lymphocyte subpopulations between humans and marmoset monkeys resulting from TCDD exposure; there are limited data to evaluate whether similar lymphocyte subpopulation alterations would result from CDF exposure.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 30: The following study was mentioned in the mechanism section previously because it deals with a potential epigenetic perturbation caused by CDFs. It is also relevant to the early life exposure being a susceptible period to the imprinting of an individual.

Reference: Su KY, Li MC, Lee MW, Ho BC, Cheng CL, Chuang YC, Yu SL, Guo YL. Perinatal polychlorinated biphenyls and polychlorinated dibenzofurans exposure are associated with DNA methylation changes lasting to early adulthood: Findings from Yucheng second generation. Environ. Res. 170: 481-486 (2019 March).

RESPONSE: *As noted in the Response to Comment 20, the results of Su et al. (2019) study were considered preliminary and were not added to the profile.*

COMMENT 31: The following reference gives concentrations of a variety of CDFs in a non-human primate both in the adult as well fetus. It reports values in a variety of tissues that are targets of toxicity both in the fetus as well as the adult. It reports that the half-life of 2,3,7,8-tetraCDF in fetal adipose tissue is significantly longer than the half-life in adult adipose tissue. Also, it shows that PCDFs do not accumulate in the fetal liver (less than one tenth) like they do in the adult liver (presumably because there are significantly reduced amounts of the Cyp1a2 binding protein in the fetal liver). Reference: Hagenmaier H, Weismüller T, Golor G, Krowke R, Helge H, Neubert D. Transfer of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) via placenta and through milk in a marmoset monkey. Arch. Toxicol. 64(8): 601-615 (1990); doi: 10.1007/BF01974688.PMID: 2128593.

RESPONSE: *The results of the Hagenmaier et al. (1990) study were added to Section 3.1.2:*

A study in marmoset monkeys compared tissue concentrations of CDF congeners in maternal and offspring tissues (Hagenmaier et al. 1990). The concentrations of pentaCDF, hexaCDF, heptaCDF, and octaCDF congeners were considerably lower in fetal (pooled sample from 18-week twins) liver tissue compared to maternal liver tissue. For example, fetal concentrations of 2,3,4,7,8-pentaCDF, 1,2,3,6,7,8-hexaCDF, and 1,2,3,4,6,7,8-heptaCDF were approximately 30, 300, and 700 times, respectively, lower than maternal concentrations. At birth, CDFs concentrations in the liver were much lower than adults exposed for a similar duration; the sum of CDF congener concentrations were 1,059 pg/g wet weight in the newborns compared to 54,584 pg/g wet weight in adults. However, the concentration of CDF congeners in adipose tissue were similar in the newborn and adult monkeys. On PND 33, the sum of CDF congener concentrations in infants was approximately 4 times lower than in adults (20,266 versus 87,929 pg/g wet weight). The largest differences were found for the higher chlorinated congeners; the ratios of infant to mother were 0.1, 0.2 and 0.7 for octaCDF, heptaCDF, and hexaCDF, respectively. In contrast the tetraCDF and pentaCDF congener concentrations in the liver were similar in the infants and adults (ratios of 1.1 and 1.0, respectively) (Hagenmaier et al. 1990).

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 32: The section of the document that discusses susceptible populations primarily focuses on different developmental stages such as during embryogenesis. If the process referenced in the previous question is important in the toxicological response to CDFs, then it might be logical to suggest that the two periods during embryogenesis when the embryo demethylates the genome and remethylates it could be sensitive periods.

RESPONSE: *As noted in the Response to Comment 30, the Su et al. (2019) study of DNA methylation was not added to the profile.*

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 33: The biomarkers of exposure that are mentioned in the document are essentially analytical measuring the CDFs themselves. In this case, they are clearly specific for the substance.

RESPONSE: *No response needed.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 34: There are a number of biomarkers of effect that are listed in the document. The four that are enumerated include chloracne, changes in the Meibomian glands of the eyelids, decrease of placental EGF receptor, and the caffeine breath test. All of these endpoints suffer from non-specificity because chemical compounds other than the CDFs can also induce these endpoints. However, the caffeine breath test may have additional issues. " In this test, [^{13}C-methyl] caffeine is ingested by subjects, and hepatic cytochrome P-450IA2-dependent caffeine 3-N-demethylase activity is monitored by determining the amount of caffeine exhaled as radiolabeled CO. The CBT is not specific for CDFs since CDDs, PCBs, and other polyaromatic hydrocarbons also induce cytochrome P-450IA." (CDF document). The additional problem for the caffeine breath test is that the enzymatic activity that is being monitored in

this assay is supplied by Cyp1A2, which is the same protein that serves as the intracellular binding protein in the liver for CDFs.

RESPONSE: *Section 3.3.2 was revised to delete the discussion of the caffeine breath test since it is not specific to CDFs.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 35: There is discussion of three types of interactions between chemical compounds in the document. The first interaction refers to the TEQ approach where various chemicals that have a similar mechanistic process as TCDD can each be additively assessed by determining the concentration of the compound and multiplying it by a factor that represents the affinity of the compound for the AhR receptor. The second type of interaction is where a relatively non-potent AhR agonist is at such a high concentration that it blocks a more potent agonist from interacting with the receptor, thus leading to an antagonism. The third type of interaction is where an animal has been treated with a mutagenic initiator (in this case MNNG) and then the administration of CDFs can serve a promoter role thus enhancing the carcinogenic process. This process can occur irrespective of which chemical compound serves as the mutagenic compound that can serve as the initiator (there are a variety of chemicals that are potent chemical mutagens). Post-initiation exposure to the promoter then enhances the induction of the cancer process. All three of these types of interactions are characteristic of chemical interactions that might occur at hazardous waste sites.

RESPONSE: *The types of interactions noted by the Reviewer are discussed in Section 3.4.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 36: There are a variety of chemical substances that could be theoretically predicted to interact with the CDFs. For example, polyaromatic hydrocarbons (PAHs) are compounds that are bioactivated by Cyp enzymatic activity that is induced by the activation of the AhR (as occurs when the CDFs serve as ligands for this receptor).

From a theoretical perspective, there are some populations with chemical exposures that may be highly susceptible to the activation of the Ah receptor. For example, there is crosstalk between the nuclear estrogen receptors and the Ah receptor, such that CDFs may activate estrogen sensitive genes and vice versa. Thus, it is possible that estrogenic endocrine disruptor compounds may interact with AhR agonists.

A publication that shows that the TEQ approach of additive interactions may have difficulties is as follows: Nagao T, Golor G, Hagenmaier H, Neubert D. Teratogenic potency of 2,3,4,7,8-pentachlorodibenzofuran and of three mixtures of polychlorinated dibenzo-p-dioxins and dibenzofurans in mice. Problems with risk assessment using TCDD toxic-equivalency factors. Arch. Toxicol. 67(9): 591-597 (1993). doi: 10.1007/BF01974065.PMID: 8311685

RESPONSE: *It is outside of the scope of the toxicological profile to discuss the validity of WHO's TEF approach. Section 3.4 was revised to include Nagao et al. (1993) as an example of a study evaluating TEFs:*

> The validity of the TEF approach for assessing mixtures of CDFs and CDDs has been investigated using both environmental (Eadon et al. 1986) and experimental mixtures (DeVito et al. 1993; Pluess et al. 1988a) with varying results depending upon the endpoint assessed (Eadon et al. 1986; Nagao et al. 1993; Pluess et al. 1988a).

Chapter 4

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 37: I assume that all of the information is correct (although I have not performed an extensive search to check if that is the case). There are many values in Table 4-2 (Physical and Chemical Properties of CDFs) for which there is no data. Further information regarding these basic parameters on the CDFs would be welcome by the scientific community.

RESPONSE: *ATSDR agrees that additional data on physical and chemical properties for CDF congeners would be useful; this is identified as a data need in Section 6.2.*

COMMENT 38: It would be helpful if the category of solubility: organic solvents could be more quantitative. In this category, there are responses that give an organic solvent for which the specific CDF congener is soluble. However, it would be helpful to researchers and chemists if a quantification of the solubility in the specific organic solvent could be given. For example, if a congener is soluble in ethanol, then specify that the compound is soluble in ethanol to 120 mM.

> Reference: Datta S, Limpanuparb T. Quantum chemical investigation of polychlorinated dibenzodioxins, dibenzofurans and biphenyls: Relative stability and planarity analysis. Molecules 25(23): 5697 (2020 Dec 3). doi: 10.3390/molecules25235697.PMID: 33287203

RESPONSE: *ATSDR guidance for development of the toxicological profiles does not require the quantification of solubility in specific organic solvents.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 39: Although the Table 4-2 with basic physical and chemical information on specific CDF congeners has numerous physical and chemical parameters for which there is no data. For example, for the octanol-water partition coefficient there are values for 7 of the 16 congeners that are listed in the Table. The Table only shows values for 16 of the 87 congeners that have four or more chlorine atoms in the structure. It would be valuable (but difficult according to the text) to get information on the parameters for which there is no data.

RESPONSE: *As noted in Section 4.1, Tables 4-1 and 4-2 include the 2,3,7,8-substituted congeners (known or suspected to be the most toxic) and other congeners for which there are health effects data. ATSDR agrees that there a number of data gaps in chemical and physical properties; these data needs have been identified in Section 6.2 (see Response to Comment 37).*

Chapter 5

COMMENT 40: The topic of this Chapter is not specifically within my expertise. I read the Chapter and found it to be very thorough, but I am not immersed in this literature.

RESPONSE: *No response needed.*

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 41: The information on production, import/export, use and disposal of the substance is relatively complete. In essence the compound is no longer being produced except in small quantities for experimental purposes.

Reference: Dharmarathne NK, Mackie JC, Kennedy EM, Stockenhuber M. Thermal oxidation of dieldrin and concomitant formation of toxic products including polychlorinated dibenzo-p-dioxin and dibenzofuran (PCDD/F). Chemosphere 225: 209-216 (2019 Jun).

RESPONSE: *The Dharmarathne et al. (2019) paper was not considered relevant to the profile since it is an experimental examination of the decomposition products of dieldrin.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 42: The text provides detailed information regarding the production of CDFs and the quantities found in various environmental samples. From the information in the document it is possible to determine what sources are leading to human exposures.

RESPONSE: *No response needed.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 43: The document extensively documents transport, partitioning, transformation, and degradation of CDFs in non-biologic processes.

RESPONSE: *No response needed.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 44: The text does provide information on the levels of these compounds in various environmental compartments. One problem is that the compounds are relatively hydrophobic and as a result the concentrations of these agents in water are very low and consequently close to the limit of detection. As a result, scientists have had more success analyzing for the compounds in either sediment or organisms where the compounds are at higher concentrations than they are detected in water.

RESPONSE: *No response needed.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 45: The report describes sources and pathways of exposure for the general population, occupations with the potential for increased exposures as well as populations that may receive excessive exposures. It is my interpretation that the potential for excessive exposures is handled comprehensively.

RESPONSE: *No response needed.*

Chapter 6

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 46: I am unaware of studies or references that can fill a data gap.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 47: The section on identification of data needs does a nice job of identifying data that is scientifically relevant to understanding the toxicology of the polychlorinated dibenzofurans. However, from a societal perspective, it is difficult to determine how much of this information is actually necessary to handle the environmental risks associated with this category of compounds. First and foremost, these compounds are no longer being synthesized in quantities that are causing large segments of the population to be exposed to the chemical compounds. Consequently, the levels of these compounds detected in humans in the NHANES assessments are constantly declining. What this probably means is that the biological endpoints are going to need to be refined in order to assess more specific pathologies associated with this family of molecules. So how much scientific effort should go into assessing all of the potential toxicological endpoints of these chemicals? For example, there were no identified toxicological studies where the CDFs were administered by the respiratory route. Although to understand exactly what would happen by giving CDFs by this route probably has some relevance. Is it necessary to give each of the CDF congeners by the respiratory route to get a full understanding of the toxicological profile of this class of compounds? Especially because it is projected that the vast majority of human exposure occurs from ingestion. From my perspective, the bioavailability of CDFs by the inhalation route will probably be comparable to the bioavailability of the compounds given by the oral route (because that is the case for 2, 3,7, 8-TCDD). If there was an experiment with the most potent (or one of the most potent CDFs) given by the inhalation route, then it may not be so important to examine all of the potential congeners by this

particular route of administration. Perhaps if there is going to be a category of compounds that will receive extensive toxicological testing on all the various forms of the compound, then CDFs are not the category to perform such extensive assessments.

This is not to say that some of the identified data needs are not very important. For example, collecting accurate data on the chemical and physical properties of the CDFs would be one data need that should be filled. Also, perhaps further information on carcinogenesis might be deemed relevant because the regulatory assumption is that extremely small doses of carcinogens can still induce cancer in a small segment of the population. It may also be deemed important to determine which endpoints are the most sensitive to a specific CDF for the purpose of establishing an MRL. In any of these circumstances, it may be deemed important to generate data that will provide an answer to a toxicological question. Thus, the suggestion is to be judicious in determining our needs with respect to these compounds.

RESPONSE: *The intent of Section 6.2 is to identify data gaps and needs. It does not prioritize the need for studies to address the data gaps.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 48: There does not appear to be any bias in the presentation of the material.

RESPONSE: *No response needed.*

Chapter 7

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 49: I am not aware of any additional regulations or guidelines relating to this category of chemical agents. However, my area of expertise is in the scientific aspects of toxicology and therefore is quite removed from the area of laws, regulations, and guidelines established by various agencies.

RESPONSE: *No response needed.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 50: I am not aware of any regulations or guidelines that should be removed. There is a single entry in Table 7.1 that seems to contradict other sections of the report. Under the cancer category there is an entry for IARC that states that 2,3,4,7,8-pentaCDF is a group 1 carcinogen. At other places in this document, it has been stated that as a category CDFs are rated as group 3 carcinogens. There does not appear to be any discussion of this issue in this document.

RESPONSE: *The text in Section 1.2 and 2.19 has been revised to reflect IARC's cancer classification for 2,3,4,7,8-pentaCDF.*

Section 1.2:

The International Agency for Research on Cancer (IARC 2012) concluded that 2,3,4,7,8-pentaCDF is carcinogenic to humans; the agency also concluded that other CDF congeners are not classifiable as to their carcinogenicity to humans (Group 3) (IARC 1997).

Section 2.19:

IARC (2012) concluded that 2,3,4,7,8-pentaCDF is carcinogenic to humans (Group 1). Other CDF congeners are not classifiable as to their carcinogenicity to humans (Group 3) (IARC 1997).

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT 51: The appendices are acceptable and helpful.

RESPONSE: *No response needed.*

Annotated Comments on the Toxicological Profile

COMMENT 52: Regarding the statement in Section 1.1**--** three important processes that account for the majority of unintentional production of these substances: (1) thermal reactions such as releases—the Reviewer made the following comment "(Is this referring to hazardous waste facilities? Otherwise, there is no mention of hazardous water incineration facilities in the document.)

RESPONSE: *Section 5.3 includes a discussion of thermal reactions, which includes waste incineration. The release of CDFs resulting from incineration is also discussed in several other sections of the profile including Section 5.1, 5.2.1, 5.5.1, and 5.5.3.*

COMMENT 53: Regarding the statement in Section 1.2--Most of the information on human health effects that pertains to CDFs is from studies of people who ingested contaminated rice oil for up to 9–10 months during the Yusho and Yu-Cheng poisoning incidents in Japan and China—the Reviewer crossed out China and replaced it with Taiwan.

RESPONSE: *The suggested revision was made in Section 1.2:*

Most of the information on human health effects that pertains to CDFs is from studies of people who ingested contaminated rice oil for up to 9–10 months during the Yusho and Yu-Cheng poisoning incidents in Japan and Taiwan.

COMMENT 54: Regarding the Figures 1-1 thru 1-4, the Reviewer commented "*(Would Figures 1-1 thru 1-4 benefit from listing the species in which the endpoint was detected?)"*

RESPONSE: *Figures 1-1 through 1-4 are high- level visual summaries of the available toxicity data, specifically the lowest doses that particular health effects are observed. It intentionally lacks specific study details, such as species.*

COMMENT 55: Regarding the statement in Section 1.2**--** The International Agency for Research on Cancer (IARC 1997) concluded that CDFs are not classifiable as to their carcinogenicity to humans (Group 3), the Reviewer commented "In Table 7-1 of the current document it is stated that IARC categorizes 2,3,4,7,8-pentaCDF as group 1 carcinogen."

RESPONSE: *Section 1.2 was revised to indicate that IARC has classified 2,3,4,7,8-pentaCDF as a human carcinogen:*

The International Agency for Research on Cancer (IARC 2012) concluded that 2,3,4,7,8-pentaCDF is carcinogenic to humans; the agency also concluded that other CDF congeners are not classifiable as to their carcinogenicity to humans (Group 3) (IARC 1997).

COMMENT 56: Regarding the summary of the Moore et al. (1976, 1979) study in Table 2-2, the Reviewer commented "There is no C57BL/6F"

RESPONSE: *As noted in Moore et al. (1979), the mouse strain was C57BL/6fh; Table 2-2 was corrected.*

COMMENT 57: Regarding Table 2-2, the Reviewer commented "In Table 2-2 there are approximately 5 entries (entry 12, 36, 38, 41, 45) which are shaded in green. Does this mean anything? I cannot find an explanation."

RESPONSE: *The following text was added to the footnote for Table 2-2:*

Principal study for an MRL

COMMENT 58: Regarding the statement in Section 2.6-- Intermediate-duration exposure also resulted in gastric mucosal changes in rhesus monkeys treated with dietary 2,3,7-tetraCDF for 2 or 6 months (McNulty et al. 1981)—the Reviewer commented "(missing a number)"

RESPONSE: *The typographical error in Section 2.6 was corrected:*

Intermediate-duration exposure also resulted in gastric mucosal changes in rhesus monkeys treated with dietary 2,3,7,8-tetraCDF for 2 or 6 months (McNulty et al. 1981).

COMMENT 59: Regarding the discussion of mechanisms in Section 2.13, the Reviewer corrected the acronym for 5′-diphospho-glucuronosyltransferase from UDGPT to UDPGT

RESPONSE: *The typographical error in Section 2.13 was corrected:*

CDFs induce hepatic uridine 5′-diphospho-glucuronosyltransferase (UDPGT) likely through an Ah receptor-mediated pathway (Ross et al. 2000). Exposure of rats to 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, or 2,3,4,7,8-pentaCDF resulted in decreases in serum T4 levels and increases in UDPGT activity (Ross et al. 2000). However, the magnitude of the decrease in T4 was not directly related to the increase in UDPGT activity.

COMMENT 60: Regarding the discussion of 1,2,3,7,8-pentaCDF immunological effects in Section 2.14, the Reviewer commented "(No species listed, although it is assumed to be rats from the previous entry.)"

RESPONSE: *The text in Section 2.14 was revised:*

A 13-week exposure of rats to 20 µg/kg/day 1,2,3,7,8-pentaCDF resulted in decreases in thymus weight and minimal thymic atrophy (Pluess et al. 1988a). No alterations were observed in the spleen.

COMMENT 61: Regarding the statement in Section 2.21**--** Oxidative stress via DNA single strand breaks tissues in rats administered via gavage 2,3,4,7,8-pentaCDF 5 days/week for 13 weeks (Hassoun et al. 2000)—the Reviewer commented "(does ROS result from ssDNA breaks?)"

RESPONSE: *The Hassoun et al. (2000) study demonstrated significant increases in reactive oxygen species. However, the study authors also suggested that other mechanisms may have contributed to the increase in oxidative stress. The text in Section 2.21 was revised:*

Oxidative stress, which may have resulted from DNA-single strand breaks, was examined in hepatic and brain tissues in rats administered via gavage 2,3,4,7,8-pentaCDF 5 days/week for 13 weeks (Hassoun et al. 2000).

COMMENT 62: Regarding the statement in Section 3.1.1-- Relative bioavailability was measured as the ratio of tissue congener levels following oral dosing with the congener in soil or in a reference vehicle (soil/reference), typically corn oil or some other lipid—the Reviewer commented "(This contrasts with the classic definition of bioavailability)"

RESPONSE: *This is referencing the bioavailability of CDFs from soil.*

COMMENT 63: Regarding the statement in Section 3.1.2-- Four days following an oral dose of ^{14}C-labeled 2,3,4,7,8-pentaCDF, approximately 50% of the ^{14}C dose was in liver, 4–5% was in adipose, ~1% was in skin, and 1% was in skeletal muscle (Diliberto et al. 1999)—the Reviewer commented "If this refers to mice, what strain?"

RESPONSE: *The strains were added to the referenced statement in Section 3.1.2:*

Four days following an oral dose of ^{14}C-labeled 2,3,4,7,8-pentaCDF, approximately 50% of the ^{14}C dose was in liver, 4–5% was in adipose, ~1% was in skin, and 1% was in skeletal muscle of C57BL/6N and 129/Sv mice (Diliberto et al. 1999).

COMMENT 64: Regarding the statement in Section 3.1.2-- After six or seven weekly doses of 2,3,7,8-tetraCDF at 1 µg/kg, the distribution of ^{14}C in the tissues of guinea pigs was similar to that observed after a single oral dose (Decad et al. 1981a)—the Reviewer asked "What route?".

RESPONSE: *The text in Section 3.1.2 was revised to indicate that it was 6–7 weekly oral doses:*

Liver, muscle, and skin accounted for ≈16% each. After six or seven weekly oral doses of 2,3,7,8-tetraCDF at 1 µg/kg, the distribution of ^{14}C in the tissues of guinea pigs was similar to that observed after a single oral dose (Decad et al. 1981a).

COMMENT 65: Regarding the statement in Section 3.4-- Therefore, it is of vital importance to elucidate whether interactions occur and what is their nature, so that toxicity of mixtures is appropriately estimated, including mixtures associated with hazardous waste sites as well as the Yusho and Yu-Cheng incidents—the Reviewer commented "(Laudable goal, no current solution)"

RESPONSE: *No response needed.*

COMMENT 66: Regarding the statement in Section 3.4**--** These results suggest that antagonist activity depends on the strain and the relative concentration ratios of agonist and antagonist—the Reviewer commented "(Due to experimental design it is not possible to know if the results are due to dose, strain, or both.)"

RESPONSE: *The text in Section 3.4 was revised to indicate that this was the investigators' conclusion:*
The investigators suggested that the antagonist activity depends on the strain and the relative concentration ratios of agonist and antagonist.

COMMENT 67: Regarding the statement in Section 3.4-- Mice pretreated with acetone or MNNG followed by 3.3 or 33.3 µg/kg/day 1,2,3,4,7,8-hexaCDF, respectively, experienced 35% mortality compared to 0% in controls—the Reviewer commented "(I was unable to determine dose based on dose per mouse. What assumptions? These doses apply to many of the subsequent paragraphs.)"

RESPONSE: *The doses were calculated using the dose per mouse and an assumed body weight of 0.03 kg.*

COMMENT 68: Regarding the statement in Section 5.2.4**--**Investigators have postulated the following combustion criteria for land-based incineration of CDF wastes: a 2-second dwell time at 1,200°C or 15-second dwell time at 1,600°C—the Reviewer commented "(a longer time and higher temperature to get same effect?)"

RESPONSE: *This is reporting experimental data from tests done using different conditions.*

COMMENT 69: Regarding the statement in Section 5.6-- Therefore, it was concluded that partitioning of higher chlorinated CDFs is not dependent on lipid content, but on specific binding to the protein fraction of serum and whole blood (Patterson et al. 1989; Schecter et al. 1991c)—the Reviewer asked "(Which proteins in serum are not mentioned in the previous toxicology sections)"

RESPONSE: *The studies measured CDF levels in the serum protein fraction; they did not include an analysis of specific binding protein.*

COMMENT 70: Regarding the statement in Section 6.2 (Absorption, Distribution, Metabolism, and Excretion)--Studies regarding distribution through the placenta after inhalation and dermal exposures were not available—the Reviewer asked "(Are such studies necessary? Transplacental distribution derives from the blood.)"

RESPONSE: *The referenced statement was deleted from Section 6.2.*