FINAL REPORT TO AUSTRALIAN GOVERNMENT DEPARTMENT OF VETERANS' AFFAIRS

Levels of 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin in Australian Vietnam Veterans compared to the Australian population

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22 December 2008

Executive Summary

Compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are formed in combustion processes and released to the environment from incinerators and other sources. TCDD was also a contaminant in the herbicide 2,4,5-trichlorophenoxyacetic acid, which was widely used in the US, New Zealand, and other countries in the 1950s and 1960s, and later. Recognition of these and other sources of dioxins have led to actions to reduce or eliminate the release of these compounds to the environment. Exposure to TCDD and other chlorinated dioxin and furan compounds is ubiquitous in the general environment. Agent Orange and other herbicide mixtures sprayed in Vietnam contained TCDD as a specific contaminant.

Due to the persistence of TCDD and related compounds, in theory, elevations of TCDD in blood in veterans compared to the general population could be used as a marker of exposure to Agent Orange, even in samples taken years after last date of service. Exposure to TCDD has been suggested to be associated with diabetes mellitus although the results of epidemiological studies do not provide a conclusive answer regarding this association. It has been suggested that based on available evidence high exposure to TCDD may cause a long term change in glucose metabolism and thus there is a possible link between exposure to TCDD and diabetes;

In this study we firstly provide an update/summary of recent publications that assessed the association between diabetes and exposure to dioxins. In addition we assessed the results of de-identified chemical analysis data from 102 Veterans obtained from the Department of Veteran Affairs to address three questions:

1. Are the measured concentrations of TCDD in the sample of 102 Australian veterans elevated compared to the Australian general population of the same age range?
2. Do the data suggest a selective elevation of TCDD, which could be a marker of Agent Orange exposure?
3. Are the measured concentrations of TCDD in these veterans in excess of the upper bound of background TCDD concentrations in the general Australian population of the same age range?

Firstly, we found that the interpretation of the data is complicated by the variability of the quality of the available analytical data with a highly variable limit of quantification. In fact in a few samples the level of reporting was higher than the highest detected concentration.

For the interpretation we compared the analytical results from the Veterans with those from Australia's National Dioxin Program. The expected concentration of TCDD in > 45 year old males in Australia in samples collected in the 2002/03 period are 1.1 pg /g lipid (45 – 60 years) and 1.3 pg/g lipid for > 60 year old males. In contrast, the TCDD concentrations obtained in the 58 Vietnam Veterans with detectable levels is more than a factor two greater. However other congeners that are not associated with contamination of herbicides were similarly elevated suggesting that the elevated mean concentration is not related to exposure to Agent Orange. Furthermore using the variability of TCDD concentrations in studies on individuals from the US we can

expect that 95 % of the data is within a factor of 4 from the mean. In the DVA data all detectable TCDD concentrations were < 7 pg/g lipid and only two data points were above 5 hence our data are not dissimilar to what may be expected for the general population (i.e. NDP data).

Based on the existing literature we considered back extrapolation of the results. We then provide an evaluation of the assumptions that need to apply to make such a back extrapolation valid and conclude that due to the low observed/measured TCDD concentration which are not statistically different from background concentrations plus the fact that both TCDD AND other PCDD/PCDF are elevated such a back extrapolation would be misleading and not meaningful. Hence a discussion of this is provided but no back extrapolation is carried out.

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1. Background

1.1 Serum Dioxins and Furans as Markers for Exposure to Agent Orange

Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other chlorinated dioxin and furan compounds is ubiquitous in the general environment. Such compounds are formed in combustion processes and released to the environment from incinerators and other sources. TCDD was also a contaminant in the herbicide 2,4,5-trichlorophenoxyacetic acid, which was widely used in the US, New Zealand, and other countries in the 1950s and 1960s, and later. Recognition of these and other sources of dioxins have led to actions to reduce or eliminate the release of these compounds to the environment. Due to their significant persistence in the environment and in the human body, these compounds can be found in the blood of persons in the general population all over the world, and hence in general, the concentrations of these chemicals is higher in older compared with younger groups of the population (i.e. Harden et al. 2004).

Agent Orange and other herbicide mixtures sprayed in Vietnam contained TCDD as a specific contaminant, generally without significant levels of other dioxin or furan compounds. TCDD is highly persistent in humans, with an estimated elimination half-life of approximately 5 to 9 years (reviewed in Milbrath et al., in press). These elimination half-life estimates are based on evaluation of serial measurements over time of blood concentrations in individuals with elevated serum concentrations, usually in workers involved in the manufacture of trichlorophenol and 2,4,5-trichlorophenoxyacetic acid. The elevated concentrations in these individuals allowed researchers to neglect the impact of ongoing, dietary exposure to TCDD in their estimations. However, as noted by several researchers, as measured serum levels fall towards background levels (which reflect exposures in the general population), the apparent half-lives will lengthen, because observed changes in concentration reflect both eliminated compound and recent intakes (van der Molen et al. 1998). As serum levels decrease, the absolute amount of TCDD eliminated per year becomes smaller, and the magnitude of ongoing intake becomes non-negligible compared to the eliminated amount of chemical. The net effect is an apparent slowing of elimination.

Other impacts on apparent elimination rates have also been noted and reviewed in a recent publication (Milbrath et al., in press). Elimination half-lives tend to increase with increasing age, are somewhat shorter for smokers than for non-smokers, and can also increase with increasing body fat. For women, current or recent breastfeeding activity will increase elimination rates (i.e. decreases half-lives). Finally, there is substantial inter-individual variability in elimination rates.

Due to the persistence of TCDD and related compounds, in theory, elevations of TCDD in blood in veterans compared to the general population could be used as a marker of exposure to Agent Orange, even in samples taken years after last date of service. Studies of Vietnam veterans in the US have demonstrated that, while some veterans displayed elevated TCDD concentrations in blood samples taken 15 to 30 years following return from Vietnam, most veterans did not (CDC 1988). Elevated

concentrations were most likely to be found in veterans with assignments to units that directly handled or sprayed herbicides containing 2,4,5-T. However, even among those units, many veterans did not display elevated TCDD concentrations in blood samples taken years after their return. For example, in the Ranch Hand study blood samples taken in 1987 (a minimum of 15 years following end of service in the Ranch Hand unit), more than 40% of the samples had TCDD levels below 10 ppt (upper bound of US background levels at that time) (see, for example, Michalek et al. 1999).

1.2 Relationship Between Dioxin Exposure and Diabetes

Primary diabetes is a metabolic disorder characterized by hyperglycaemia and qualitative and/or quantitative deficiencies of insulin action. In addition to gestational diabetes that occurs during pregnancy the two main types of diabetes that are recognized in males are:

- Type 1 diabetes (~10 % of cases) which is insulin dependant diabetes (IDDM) and results from beta-cell dysfunction caused by a genetically based autoimmune destruction. Type 1 diabetes typically occurs with an early onset in youth although it can form also in later stages in life. Environmental factors that may trigger onset in genetically susceptible individuals have been proposed.

- Type 2 diabetes is far more common than Type 1 diabetes. Type 2 diabetes is referred to also as non-insulin dependant diabetes (NIDDM). Its onset is characterized by insulin insensitivity and it is reversible for example by addressing diet and activity but also through medication that improve insulin sensitivity. In contrast to Type 1 diabetes it typically commences later in life (typically > 30 years old) with an increase in risk with increasing age. It is noteworthy however that Type 2 diabetes is also increasingly affecting children. In addition to age, recognized risk factors include among others central obesity (fat concentrated around the waist), physical inactivity as well ethnicity and family history of the disease.

1.2.1 Epidemiological Studies

The evaluation of association between diabetes mellitus and exposure to TCDD or other POPs has primarily focused on epidemiological studies including cohorts such as Vietnam Veterans, occupational exposed groups or accidentally contaminated cohorts such as residents in Seveso or the Yucheng victims. Some of the problems with epidemiological studies and particularly evaluation of diabetes relates to the diagnosis of the disease (i.e. non-differential misclassification) where it has been pointed out that the diagnostic accuracy of death certificates for diabetes is low (Consonni et al. 2008).

Recent expert reviews have been presented by Gaus et al. (2004) in a report for the Government of Western Australia, and included reviews by the following groups:

- The ***International Agency for Research on Cancer*** (IARC) Review 1997 – summarized 5 studies (including 2 Vietnam Vet cohort studies). IARC provided no conclusion on the association between dioxins and diabetes.
- The ***Agency for Toxic Substances and Disease Registry*** (ATSDR) 1998 reviewed 5 studies including 2 Vietnam Veteran studies but also the Seveso cohort

study. ATSDR concluded that available evidence suggests that at high exposure TCDD may cause a long term change in glucose metabolism. The ATSDR review suggested TCDD may cause adverse effects.

- The ***US-Environmental Protection Agency*** (2000) in a draft document evaluated epidemiological studies including those of Vietnam Veterans but also occupationally exposed cohorts. Based on this review the US-EPA concluded that at high exposure, glucose metabolism may be affected and thus there is a possible link between exposure to TCDD and diabetes;
- The ***Institute of Medicine*** (2003, 2005, and 2007) updated their "Veterans and Agent Orange" review on the health effects of Agent Orange and continued to place type 2 diabetes into a category of outcomes with 'limited or suggestive evidence' of an association between exposure to dioxins (and/or related herbicides) and the outcome. The IOM committee does not draw conclusions regarding whether the observed associations are causal, noting: "Such factors as consistency of evidence, biologic plausibility, temporality, dose-response relationships, and strength of association may be considered when deciding whether an observed statistical association is causal. The committee's charge, however, did not extend to making determinations of causality, so it drew no conclusions regarding cause-and-effect relationships." (IOM 2006, p. 35).

The literature examining potential relationships between dioxin exposures and diabetes occurrence is extensive. A variety of mechanistic pathways suggesting a potential impact of TCDD and related compounds on diabetes have been hypothesized and, in some cases, demonstrated, in animal and *in vitro* studies (reviewed in Remillard and Bunce, 2002). In general, these effects have been demonstrated only at exposures in animal or *in vitro* studies that are 10 to 1,000 times higher than the exposures required to produce the most sensitive responses to TCDD. As with nearly all of the other toxic effects demonstrated for TCDD, these effects appear to be mediated through binding of TCDD to the aryl hydrocarbon (AH) receptor, with a variety of responses occurring subsequent to binding, transport of the bound receptor to the cellular nucleus, and interaction between the ligand-bound receptor, cofactors, and DNA. Binding to the AH receptor requires specific structural characteristics shared by TCDD, other 2,3,7,8-substituted dioxin and furan compounds, selected PCB compounds with lateral chlorine substitution, and a variety of other natural and synthetic compounds with specific structural characteristics (reviewed in van den Berg et al. 2006). These mechanistic pathways are NOT shared by the broader class of persistent organochlorine compounds such as the vast majority of PCB compounds, DDT and related metabolites, or other persistent organochlorine compounds.

As noted by Remillard and Bunce (2002), studies of highly exposed industrial populations have generally not found increased risks of diabetes (see, for example, Steenland et al. 2001), while a number of studies of populations with lower measured serum dioxin levels (within or near the range of background exposures in the US) have found associations with diabetes or related conditions. In general, these studies have had a cross-sectional design, and issues of interpretation of causality in cross-sectional studies, including those relying upon biomonitoring data as markers for exposure, are well recognized (see, for example, LaKind et al. 2008).

Evaluation and interpretation of the potential associations between exposure to dioxin-like compounds and diabetes and related conditions in cross-sectional epidemiologic studies is complicated by the interrelationship of factors that are both risk factors for diabetes and that may influence the levels or elimination rates of dioxin-like compounds (Longnecker 2006). In particular, increased body fat levels have been shown to reduce the elimination rate of dioxin-like compounds in both rats and humans, leading to greater accumulation of these compounds in individuals with higher body fat levels (Emond et al. 2006; Flesch-Janys et al. 1996; Collins et al. 2007; Michalek et al. 1992, 1996; Michalek and Tripathi 1999). As body fat levels are also a risk factor for diabetes, these relationships may lead to a statistical association between dioxin levels and diabetes, even though this association may not be causal. Adjustment for BMI in cross-sectional studies may not adequately address the potential confounding because BMI may not be an adequate surrogate either for body fat levels (particularly across genders) or for the pattern of distribution of body fat, which influences diabetes risk (Bray et al. 2008). In addition, current BMI may not provide an adequate measure of body fat over the previous two to three decades, which would influence the long-term accumulation rate of dioxins.

Dietary habits leading to elevated body fat levels (for example, increased rates of fat consumption over long time periods) may correlate with ongoing elevated exposure to dioxin-like compounds, since the primary mode of exposure to these compounds in the general population is via accumulation in the food chain into dietary fat. Dietary exposure to dioxin-like compounds is likely also correlated with dietary exposure to other lipophilic persistent pollutants in the food chain, including polychlorinated biphenyls, DDT and its metabolites, and other chlorinated pesticide compounds such as hexachlorocyclohexane, dieldrin, lindane, and others. These compounds have also been examined for potential impacts on diabetes risk with positive associations noted in several studies (see, for example, Cox et al. 2007; Rignell-Hydbom et al. 2007; Rylander et al. 2005; Boada et al. 2007; Everett et al. 2007). However, many of the studies examining potential relationships between serum levels of dioxin-like compounds and diabetes have not examined these other persistent compounds as potential confounders. Finally, the metabolic alterations associated with the disease could conceivably lead to further alterations in elimination rates for dioxin-like compounds (Longnecker 2006), although limited attempts to test this hypothesis have not found evidence to support it (Everett et al. 2007).

As mentioned above, the epidemiologic literature on the potential relationship between dioxin exposure and diabetes was reviewed by Gaus et al. (2004). Following is a brief update of additional recent literature related to this topic.

A series of studies have been published examining the extensive data set available from the US National Health and Nutrition Examination Survey (NHANES). NHANES employs a complex, stratified, probability-based sampling strategy of the civilian, non-institutionalized US population and collects a wide range of health and demographic information from approximately 5,000 individuals per year in two-year cycles. The study has a cross-sectional design; individuals are not followed over time, but rather, each cycle represents a cross-sectional snapshot of health indices in the US. Data are collected from questionnaires, medical examination, and laboratory and clinical tests, and includes measurement of biomarker concentrations of a wide range of environmental pollutants in blood and urine. The data sets are freely

available on the internet for download and analysis (http://www.cdc.gov/nchs/nhanes.htm).

Two groups of researchers have analyzed the NHANES data to assess potential associations between serum concentrations of dioxins and other persistent organochlorine compounds and diabetes. In an initial publication, Lee et al. (2006) evaluated low level exposure to 6 (most frequently detected) persistent organic pollutants including 2 polychlorinated dibenzodioxins (hepta and octachlorinated PCDDs – but not TCDD) in 2016 adult participants of the 1999-2002 NHANES study. The authors adjusted for many factors including age, sex, BMI, and waist circumference. The authors found strongly positive association between prevalence of diabetes and lipid adjusted concentrations of ALL of these POPs, including several that are not considered to be Ah receptor agonists or otherwise "dioxin-like" from a mechanistic point of view.

These researches have conducted further, in-depth analyses of the NHANES 1999-2002 data sets to address several issues and questions identified in their first analysis. In particular, the researchers entered the various pollutants into simultaneous models (rather than examining each compound separately). In analyses examining diabetes as the outcome, they found that in the simultaneous models, neither polychlorinated dioxins nor furans were statistically significantly associated with increased odds ratios for diabetes, while selected PCBs and organochlorine pesticides were (Lee et al. 2007a). The same pattern of results, with no significant associations with dioxin compounds, was found by these researchers in a simultaneous analysis of NHANES data for associations between POPs and insulin resistance markers in non-diabetic adults (Lee et al. 2007b). Finally, the authors also examined prevalence of "metabolic syndrome," a cluster of conditions including obesity, dyslipidemia, hyperglycemia, and hypertension in adults in relationship to POPs. This cluster of conditions is considered to be a risk factor for development of diabetes. They found significant relationships between these conditions and selected PCB groupings and organochlorine pesticides, but limited to no association with dioxin or furan levels, except for a small association with hypertension (Lee et al. 2007c).

A separate group of researchers conducted a similar analysis of the NHANES 1999-2002 data set and reported that while they found positive associations between total diabetes (diagnosed and undiagnosed) and PCB 126 or DDT, they found no significant relationship with 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin in the simultaneous model (Everett et al. 2007). The co-occurrence and often, covariance, of a wide variety of persistent organochlorine compounds in humans (with different mechanisms of action) complicates the evaluation of associations between these compounds and chronic health outcomes in cross-sectional studies, and many studies fail to evaluate a comprehensive list of these pollutants in their analyses.

Uemura et al. (2008) evaluated the association between diabetes and dioxin concentration in serum in a random sample of Japan's general population. Using a logistic regression analysis they found that, after adjustment for age, BMI, sex, smoking, and residential area, subjects in the highest quartile of PCDD/F, dioxin-like PCBs, and total TEQ had statistically significantly elevated odds ratios for prevalent diabetes with adjusted odds ratios of 2.21, 6.82, and 3.81, respectively, compared to the referent (first and second) quartiles. The authors also found similar results if

subjects with poor liver or renal function were excluded. No measurement or accounting for levels of other POPs was conducted.

Wang et al. (2008) studied 378 Yucheng subjects and 370 matched controls. The Yucheng population consists of individuals who consumed PCB-contaminated rice oil (and food cooked in the rice oil) over several months and who orally ingested large quantities of PCBs and the high-temperature degradation products from the PCBs including polychlorinated terphenyls, quaterphenyls, and PCDFs. Estimates of the quantity of PCBs and degradation products ingested are uncertain, but 14 years after the incident, Yucheng women still displayed levels of PCDFs and PCBs in serum 10 to more than 100 times higher than controls (Guo et al. 1997). Wang et al. (2008) reported a significantly elevated odds ratio for diabetes in women (OR = 2.1, 95% CI 1.1-4.5) but not for men after considering age, BMI, cigarette smoking, and alcohol intake. Yucheng women diagnosed with chloracne had adjusted ORs of 5.5 (95% CI 2.3-13.4) for diabetes and 3.5 (1.7-7.2) for hypertension compared with those without chloracne. Wang et al. suggest that the mechanism may involve the estrogen dependent peroxisome proliferator-activated receptor (PPAR) pathway.

Karouna-Renier et al. (2007) carried out a study on a population that was potentially exposed to a range of chemicals including PCDD/PCDFs from a wood treating company. They found that the occurrence of diabetes and hypertension exceeded the national averages (for non-Hispanic blacks); however, this comparison was not adjusted for age, BMI, or other covariates. Although a simple univariate analysis showed increased risk of hypertension with increasing serum dioxin levels, analysis of covariance taking into account age, BMI, alcohol consumption, and other covariates found no difference in serum levels of dioxins between individuals with and without diabetes, hypertension, or the other health endpoints examined in the study.

Michalek and Pavuk (2008) recently published a study in which they adjusted data from the Ranch Hand study of US Air Force service members who handled or sprayed Agent Orange in Vietnam for calendar period, days of spraying, and time spent in Southeast Asia. The authors operated on the hypothesis that earlier years of service might have entailed higher exposures than later years of service based on changes in the levels of TCDD in the herbicide formulations. The authors found that using cut-off points between 1967 and 1969 for delineating different calendar periods of service resulted in a ‘sharpened association’ between TCDD biomarkers and diabetes in Ranch Hand veterans. Fujiyoshi et al. (2006) described an analysis evaluating the relationship between various molecular markers associated with increased diabetes risk and serum dioxin levels in Ranch Hands and Comparison veterans. The authors reported associations between these markers and dioxin serum levels in both the Ranch Hands and the Comparison subjects, but overall the magnitude of effect on the various markers was similar in the two groups, despite dioxin levels that were much higher in the Ranch Hand group. The authors interpreted the results to indicate a “diabetogenic” effect of dioxins, but the results should be viewed with caution given the lack of a consistent dose-response for the various markers between the Ranch Hand and Comparison groups.

Consonni et al. (2008) reported an extensive mortality follow up study (1997-2001) of the Seveso cohort, which includes more than 45 000 residents of the contaminated areas of Seveso (including approximately 8 000 who moved into the area after the

date of the TCDD accident), and 232 000 reference subjects. The contaminated zones were divided into zones A, B, and R which represent zones with decreasing level of contamination. With respect to diabetes, the study found no excess mortality from diabetes in Zone A, but found excess mortality from diabetes in females (but not males) in Zones B and R (relative risks of less than 2).

A number of extended abstract studies presented at the annual Dioxin conferences in recent years have examined possible associations between dioxins or related compounds and diabetes in cross-sectional studies. These extended abstracts are not properly peer-reviewed, but do provide information that can be considered in the overall evaluation of evidence.

- Chang et al. (2007) assessed association between dioxin exposure and insulin resistance in residents that lived near a PCP factory in Taiwan. They found significant higher concentrations of polychlorinated dibenzo-dioxins and dibenzofuran AND high insulin resistance in subjects with diabetes. Furthermore within the study group (all potentially exposed) they found that simply being in the highest quartile of dioxin levels (>75th percentile) did not increase the odds ratio for diabetes, but that being in the highest quartile for dioxin AND having high insulin resistance resulted in elevated odds ratios for diabetes (OR = 4.35, 95 % CI = 1.76 – 11.2); odds ratios for diabetes in individuals having high insulin resistance without elevated dioxin levels were also significantly elevated (OR=2.76, 95% CI = 0.98 - 7.47, p=0.05), suggesting that insulin resistance (independent of dioxin level) was the primary risk factor for diabetes development in this analysis.

- Watanabe (2007) presented a case control study for workers (n=840) and residents (n=740) that were potentially exposed to dioxins via municipal waste incineration (either as worked in this industry or residents). Multi-regression analysis indicated that family history but also a wide variety of dioxin and PCB compound concentrations in the blood were positively associated with diabetes occurrence.

- Yamamoto et al. (2007) similarly carried out a study on 861 workers from 46 Japanese incinerators. They stress that the concentrations of dioxin-like chemicals in the studied population was not elevated compared to the normal population but found also a significant association between diabetes mellitus and the PCDD/PCDF concentration in serum.

- Noda et al. (2007) found significantly higher concentrations of PCBs (both coplanar, or “dioxin-like”, and non-coplanar) in 9 subjects with diabetes mellitus compared to 108 subjects without known diabetes in samples collected from subjects that signed up to a obesity control program in Japan. All of these compounds are highly lipophilic, and the authors acknowledged the possibility that the associations observed might be due to increased retention of the fat-soluble compounds in diabetic vs. non-diabetic individuals rather than necessarily due to a causal relationship between exposure and diabetes response.

- Haws et al. (2006) reported on an additional analysis of the Ranch Hand dataset on diabetes occurrence and serum dioxin measurements. They found a similar magnitude of diabetes risk in individuals in the top quartile of serum dioxin in the comparison group as in the top quartile of serum dioxin in the Ranch Hand group,

even though the magnitude of dioxin levels in the Ranch Hand group was substantially greater. This pattern of results indicates that the diabetes risk AND the serum dioxin levels are both influenced by other factors. This would suggest that the association between dioxin and diabetes observed in this group could be a co-association, that both serum dioxin and diabetes are influenced by another factor or factors, as discussed above.

Overall, the pattern of recent evidence shows that, when other persistent organochlorine compounds are not included in the analysis, cross-sectional studies tend to demonstrate an association between serum dioxin levels and diabetes occurrence. When other persistent organochlorine compounds are added into multivariate analyses, these associations tend to diminish and sometimes disappear. The interactions among factors including long term exposure to dioxins in dietary fat, reduced elimination rates of dioxins in persons with elevated body fat levels, the increased risk of diabetes associated with obesity and related to specific types of body fat, and covariation of dioxin levels with levels of other organochlorine pollutants, suggest caution in the interpretation of the causal importance of these associations in cross-sectional studies of this endpoint.

2. Evaluation of Serum Dioxin Data from 102 Australian Veterans

This evaluation addresses three questions:

1. Are the measured concentrations of TCDD in the sample of 102 Australian veterans elevated compared to the Australian general population of the same age range?
2. Do the data suggest a selective elevation of TCDD, which could be a marker of Agent Orange exposure?
3. Are the measured concentrations of TCDD in these veterans in excess of the upper bound of background TCDD concentrations in the general Australian population of the same age range?

Question 1 asks whether the serum measurements indicate elevated dioxin levels in the sampled veterans compared to the general Australian population. Question 2 examines specifically the measured levels of TCDD, which, if selectively elevated, might be indicative of exposure to Agent Orange during service in Vietnam. Question 3 addresses a key issue with respect to the feasibility of conducting back-extrapolations of measured TCDD concentrations. Even if the measures are elevated compared to appropriate reference values in Australians, measured concentrations must be clearly in excess of the upper bound of the comparable Australian general population groups in order for valid back-extrapolation to be conducted. If an individual's measured level is within the range of general population background concentrations, there is no reliable method available to determine if or how much serum levels were elevated in the past.

Based on the answers to these questions, we present an evaluation of the feasibility of conducting back-extrapolation of measured concentrations of TCDD to concentrations at the time of last service in Vietnam, and discuss appropriate methods for such back-extrapolations.

2.1 Are the Measured Concentrations in Veterans Elevated Compared to the Australian General Population of the Same Age?

2.1.1 Analysis of the data from Australian veterans

Analysis of the de-identified data obtained from the Department of Veteran Affairs was undertaken. In total hardcopies of 102 dioxin reports were received from DVA. For the analysis of the data an Excel based data sheet was developed that included all relevant information for each data set including date of birth of the individual, the period when they were active in the Vietnam conflict and the date of sample analysis. Data were entered and the analytical results were colour coded to specify whether a given result passed all QCQA criteria (i.e. reported as a concentration) or the data is associated with a qualifier (i.e. a limit of reporting is presented).

The initial examination indicated that substantial variations occurred in the analysis of dioxins which complicates the analysis. For example, the limit of reporting for 2,3,7,8-TCDD varied by more than an order of magnitude with fully quantified results as low as 0.91 pg/g lipid in one of the samples and then a value as high as 10 pg/g lipid which did not pass the QCQA criteria. This not only complicates the current interpretation of the results but one may wonder how the results were used in a regulatory frame work.

In a first examination of the data we only considered analytical data that were above the specific limit of reporting/detection (Table 2.1). In summary 2,3,7,8-TCDD was detected and quantified in 58 of the 102 individuals (57%) at an average concentration of 2.7 ± 1.2 pg/g lipid. It is noteworthy that that inclusion of the limit of reporting in the analysis (i.e. for the 45 results where 2,3,7,8-TCDD was not reliably detected or quantified) resulted in only a very slight increase in the mean concentration to 2.8 pg/g lipid. This may be seen as an indication that if anything the loss of data that were not quantified is likely to cause a bias towards an over-prediction of the concentrations from the detected results (i.e. it can be expected that the mean value is overall lower).

For an initial evaluation the Vietnam Veteran data are compared with the data from the National Dioxin Program blood serum study (Harden et al. 2004). Based on 20 pooled blood serum samples collected from a total of 2000 individuals in five geographical regions across Australia the expected concentration of TCDD in above 45 year old male Australian's is 1.1 ± 0.3 pg /g lipid (or 1.3 ± 0.4 pg/g lipid for > 60 year old males). Hence the TCDD concentrations obtained in the 52 Vietnam Veterans with detectable levels is more than a factor 2 greater than the average concentration around the same period in males of that age group.

While this could be used as an indication that the historic exposure still remains detectable it is notable that ALL congeners appear to be higher in the Vietnam Veteran population with ratios of the mean concentration in Vietnam Veterans compared to the >45 Australian Males typically in the order of 1.8 – 4 for congeners that were detected in more than 50 % of the samples. If the TCDD residue detected in the Veterans were related to the contamination via Agent Orange one would expect that either only TCDD (PeCDD to a lesser extent) would be elevated whereas the concentration ratio would be expected to be similar for other congeners. Hence, from this preliminary examination, and despite the elevated concentration of TCDD, no immediate evidence to suggest that the TCDD concentration is linked to the exposure in Vietnam. Intriguingly, the data to date suggests that the Vietnam Veterans have a higher body burden for all PCDD/PCDFs. This may be indicative of a different life style.

Table 2. 1 Summary of the concentration (pg/g lipid) of the 13 most commonly detected PCDD/Fs in data obtained from Vietnam Veterans and data from Australia's National Dioxin Program (NDP) Serum Blood study (20 pools of each 100 individual males older than 45). Also provided is the concentration ratio of the average concentration in the general Australian population compared to Vietnam Veterans

	NDP Study (AS) (only Male > 45 years old)			**Vietnam Veteran Data (VVD)**			
	Average	Stdev	N (% detected)	Average	Stdev	*N (% detected)*	Ratio (VVD/AS)
2.3.7.8-Tetra-CDD	1.1	0.3	16 (80%)	2.7	1.2	*58 (57%)*	2.3
1.2.3.7.8-Penta-CDD	3.0	0.6	20 (100%)	6.6	2.6	*79 (77%)*	2.2
1.2.3.4.7.8-Hexa-CDD	2.8	1.0	19 (95%)	7.2	3.6	*53 (52%)*	2.5
1.2.3.6.7.8-Hexa-CDD	19.5	5.3	20 (100%)	35.2	16.4	*99 (97%)*	1.8
1.2.3.7.8.9-Hexa-CDD	2.9	1.2	20 (100%)	9.8	5.6	*72 (71%)*	3.4
1.2.3.4.6.7.8-Hepta-CDD	27.7	6.1	20 (100%)	58.2	36.9	*94 (92%)*	2.1
OCDD	278.2	62.5	20 (100%)	443.4	292.3	*88 (86%)*	1.6
2.3.7.8-Tetra-CDF	*0.4*	*0.1*	*4 (20%)*	*2.1*	*1.0*	*14 (14%)*	*4.8*
2.3.4.7.8-Penta-CDF	2.5	0.6	20 (100%)	6.2	2.6	*94 (92%)*	2.5
1.2.3.4.7.8-Hexa-CDF	2.0	0.5	20 (100%)	4.1	1.8	*55 (54%)*	2.1
1.2.3.6.7.8-Hexa-CDF	1.8	0.4	20 (100%)	4.4	1.8	*63 (62%)*	2.5
1.2.3.4.6.7.8-Hepta-CDF	2.3	0.7	20 (100%)	10.9	22.8	*42 (41 %)*	4.8
OCDF	*2.1*	*1.4*	*4 (20%)*	*28.3*	*48.4*	*17 (17%)*	*13.4*

2.2 Do the Data Suggest a Selective Elevation of TCDD in these Veterans?

Since TCDD was a specific contaminant in Agent Orange, significant exposure to Agent Orange could theoretically be detected in individuals or a group by comparing the ratio of TCDD to other dioxin or furan congeners not associated with Agent Orange exposure. If the ratio of TCDD to one or more of these congeners was significantly elevated compared to the general population, this could indicate exposure to Agent Orange.

The two reference congeners selected were 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (1,6-HxCDD). These congeners were selected because they are frequently detected in general population samples, are highly persistent in humans (half-life estimates of similar magnitude as for TCDD) and are typically not associated with herbicide contamination or exposure. Ratios were calculated for each individual in the dataset with quantified concentrations of both congeners (valid ratios cannot be calculated when either or both of the congeners are below the limit of detection or limit of quantification).

The ratios of TCDD to these compounds in the Australian veterans were compared to the corresponding ratios in two reference datasets: the pooled samples from Harden et al. (2004) and the US NHANES 2003-2004 sampling dataset. The Australian general population data presented in Harden et al. (2004) is based on pooled samples and therefore it does not provide information on the likely variation in ratios across individuals. Thus, these ratios were also assessed in the US NHANES 2003-2004 dataset, which presents data for a representative sample of the US population. Although the absolute levels in the US NHANES dataset may differ from the absolute levels in the Australian population, the distribution of the ratios of TCDD to each of the reference congeners can provide a context for evaluating the corresponding ratios (and variation of ratios across individuals) in the Australian veterans' dataset.

Tables 2.2 and 2.3 present the ratios of TCDD to the reference congeners in the various datasets. The distribution of the ratio of TCDD to 4-PeCDF in the Australian veterans' dataset is similar to the distribution observed in the US NHANES dataset, and the mean ratios are similar to those from both the NHANES dataset and the Harden et al. (2004) Australian pooled dataset. The comparison is more mixed for the ratio to 1,6-HxCDD, with mean values in the Australian veterans' dataset somewhat higher than the ratios in the pooled Australian dataset, but lower than from the US NHANES dataset.

Overall, the comparison does not suggest a significant, specific elevation of TCDD compared to the other congeners in the Australian veterans' dataset as a whole, but comparisons based on 1,6-HxCDD are somewhat limited due to the apparent difference between average ratios in the Australian pooled data and the US NHANES dataset.

Table 2. 2 Ratio of TCDD:4-PeCDF for all individuals or pools with quantified concentrations of both congeners

Study/group	Age Group	N	Mean	Median	5th	95th
US NHANES 2003-2004	45-60	133	0.46	0.44	0.20	0.83
	60+	314	0.49	0.44	0.23	0.83
Harden et al. 2004[a]	45-60	8 pools	0.50			
	60+	8 pools	0.45			
Australian veteran data	45-60	34	0.46	0.40	0.24	0.78
	60+	22	0.40	0.36	0.22	0.76

[a] Unweighted average of statistic for each pool with quantified levels of both congeners

Table 2. 3 Ratio of TCDD:1,6-HxCDD for all individuals or pools with quantified concentrations of both congeners

Study/group	Age Group	n	Mean	Median	5th	95th
US NHANES 2003-2004	45-60	136	0.09	0.08	0.05	0.21
	60+	317	0.09	0.08	0.04	0.14
Harden et al. 2004[a]	45-60	8 pools	0.06			
	60+	8 pools	0.06			
Australian veteran data	45-60	35	0.08	0.06	0.04	0.14
	60+	24	0.07	0.07	0.03	0.21

[a] Unweighted average of statistic for each pool with quantified levels of both congeners

2.3 Are the measured concentrations of TCDD in these veterans in excess of the upper bound of background TCDD concentrations in the general Australian population of the same age range?

An initial criterion for assessing whether valid back-calculation can be conducted is whether or not the measured values exceed the range of typical background TCDD concentrations for persons of the same age in Australia at the time the samples were taken. General background population levels of dioxins in Australia were assessed by Harden et al. (2007) based on pooled blood samples collected in 2003 from different regions of Australia and for different age and sex strata. Consistent with other studies of dioxins in the blood of general population groups, the concentrations displayed a strong dependence on age, with increasing concentrations with increasing age. Since pooled samples were used, the measured concentrations provide information about the central tendency (approximately the arithmetic mean) of concentrations, but do not provide information on the inter-individual variability in dioxin levels or an appropriate upper bound for current concentrations within an age group.

Here we propose to estimate the upper bound of background concentrations in the Australian population of the relevant age group by using data from various US studies in persons of the same age range during approximately the same time period to examine the

pattern of the relationship between upper bound (95th and 99th percentiles) and central tendency (mean and median) measures of TCDD in these studies. In particular, we focus on two studies: Patterson et al. (2004) and the results from the analyses in the US National Health and Nutrition Examination Survey (NHANES). We use these data sets to characterize the ratios between the upper bounds and the central tendencies in persons in the age group of interest (45-59 and 60+ yrs). These ratios can then be applied to the age-specific estimates of the central tendency of TCDD concentration in the general Australian population based on the Harden et al. (2007) pooled sample results to estimate the upper bound of the population distribution for the relevant age groups in Australia based on the pooled data.

Studies of general population groups in the US and elsewhere have demonstrated that as age increases, both the concentrations and inter-individual variability in concentrations increase. This is consistent with current understanding of the pharmacokinetics of TCDD. As the compound is long-lived (half-life of elimination on the order of 7 to 9 years), inter-individual variations in exposure and elimination rates will result in the biggest variation in measured levels as the time period of exposure and/or elimination increases (i.e., in the oldest individuals). For example, Figure 2.1 shows the variation in measured TCDD concentrations with age among 588 persons from the general US population in samples collected between 1996 and 2001 (Patterson et al. 2004).

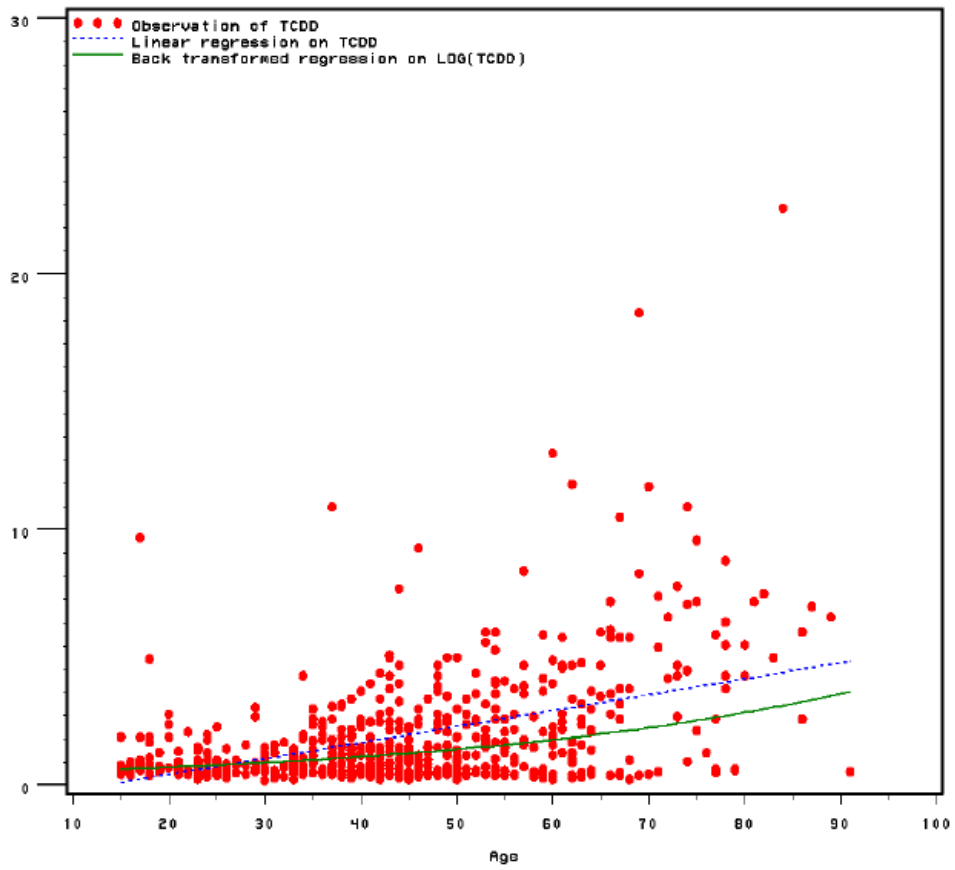

Figure 2. 1 Variation in measured TCDD concentrations (expressed as pg/g lipid) with age among 588 persons from the general US population in samples collected between 1996 and 2001 (Patterson et al. 2004).

The Patterson et al. (2004) data set is a collection of data from reference populations in four different studies of local populations; however, it is not a statistically representative sample of the US population.

The US National Health and Nutrition Examination Survey (NHANES) is an ongoing survey of numerous health and demographic outcomes using a complex, stratified, probability sample of the civilian US population. Dioxins and related compounds have been measured in a subset of the included population, and results are available for the three most recent sampling cycles: 1999-2000, 2001-2002, and 2003-2004. Due to the limited sample volume available, the detection limits for the NHANES survey are relatively high and were quite variable during the first two of these three cycles. Data from the most recently reported cycle, 2003-2004, provide the most stable data set for evaluation of the relationships between upper and central tendency measures. These data were retrieved from the US NHANES web site (http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/lab03_04.htm) and analyzed using STATA 10.0 (Stata Corp, College Station, Texas, US). Population-weighted estimates of the mean, median, 95th, and 99th percentiles were generated.

Table 2.4 provides the descriptive statistics regarding the detection and distribution of TCDD in persons in the target age range for each of the two studies.

Table 2. 4 Descriptive statistics for two recent studies of TCDD concentrations (pg/g lipid) in the general US population.

Study and Age Group	N	% Detects	Mean (SD)	Median	95th	99th
NHANES (2003-2004)						
45-<60	261	56%	2.1 (1.5)	1.8	5.2	6.6
60+	448	75%	3.8 (3.2)	3.4	6.1	7.9
Patterson et al. (2004)						
45-<60	160	NR	1.9 (1.6)	1.4	5.0	NR
60+	113	NR	3.9 (3.7)	3.2	10.9	NR

NR: Not reported

The central tendency estimates from these two studies are quite similar; however, the 95th percentile estimate for the oldest age group in the Patterson et al. (2004) dataset is substantially higher than found in the NHANES dataset. Table 2.5 provides the ratios between the upper bound and central tendency concentrations in the two studies. The greater variability observed in the Patterson et al. (2004) dataset in the oldest age group is reflected in substantially higher estimated ratios between the 95th percentile and the mean and median in this dataset compared to the NHANES dataset. Patterson et al. (2004) did not report 99th percentiles, so a similar comparison at the extreme of the data can not be made.

Table 2. 5 Ratios between estimates of the upper bound and the central tendency of serum TCDD concentrations (pg/g lipid) in two studies of the US general population.

	Ratio, 95th %ile to		Ratio, 99th %ile to	
	Mean	**Median**	**Mean**	**Median**
NHANES (2003-2004)				
45-<60	2.5	2.9	3.2	3.7
60+	1.6	1.8	2.1	2.3
Patterson et al. (2004)				
45-<60	2.6	3.6	NR	NR
60+	2.8	3.4	NR	NR

NR: Not reported

In general, upper bound levels appear to be in the range of 1.6 to 3.2 times higher than the mean. A reasonable estimated upper bound for typical general population concentrations would be approximately 3-fold greater than the appropriate age-specific mean concentration. If the degree of variation in the Australian population in these age groups is similar to that observed in the US population, then these ratios can be used to estimate a range of likely upper bounds for the Australian general population in these age groups based on the results from the analysis of the pooled samples reported in Harden et al. (2004; 2007).

The measured concentrations in pooled samples can be regarded as an estimate of the arithmetic mean concentration in the sampled population if the volume of serum included in the pool from each individual is the same. For the pooled blood samples from adults in Harden et al. (2007), this was generally true, with a 1 ml aliquot added to the pool from each contributing individual (Harden et al., 2004). The results of the analysis of pooled serum samples in males of the age range of interest are presented in Table 2.6 along with the estimated upper bound concentration associated with each pool, calculated as 3 times the measured pool concentration. The range of upper bound values associated with the pools for males 45 to 60 is from <1.2 to 3.6 pg/g lipid; for males ages >60, the range is from <2.4 to 6.3 pg/g lipid. These upper bound values are valid only for samples taken in the same general time frame as those in the Harden et al. (2004) pooled analyses. If Australian population concentrations are declining with time (as observed in other nations with regulatory efforts in place to reduce incineration emissions), the upper bound range of general population levels might be expected to be somewhat higher in samples taken prior to 2003, and lower in samples taken since 2003. However, given the relatively slow elimination of TCDD, these screening values should be appropriate for data collected between approximately 2000 and 2006, a time frame that encompasses nearly all of the sampling for the 102 Australian veterans.

The purpose of classifying individuals as within or above the range of background is to identify individuals for whom back-extrapolation to estimate concentrations at the time of last service in Vietnam may be appropriate. As the validity of such back-extrapolations depends first on a clear separation between the measured level to be back-calculated and the range of general population background levels (which reflect historical and ongoing background exposures), the high end of the upper bound estimates will be most appropriate for use to discriminate elevated levels from background levels. Rounding to whole values, this suggests a cut-off point of approximately 4 pg/g lipid for individuals aged 45 to 60 and approximately 6 pg/g lipid for those over age 60. Individuals with measured concentrations

exceeding these age-specific bounds can be classified as exhibiting measured concentrations in excess of the anticipated range of general background concentrations for their age group. Other individuals with measured levels below these cut-off points may have had elevated TCDD levels in the past, but the decrease over time to levels within the general population range makes it impossible to conduct any valid back-extrapolations for those individuals (Steenland et al. 2001a; 2001b).

Table 2. 6 Pooled serum TCDD concentrations (pg/g lipid) for males aged 45-60 and >60 yrs from Harden et al. (2004). The upper bound of background serum TCDD concentration based on each pool was estimated as 3 times the mean.

Age group	Region	Pool #	TCDD ppt, lipid adjusted	Estimated upper bound, ppt, lipid adjusted
Ages 45-60				
	South	1	1	3
		2	0.79	2.37
	Rural	1	0.99	2.97
		2	1.1	3.3
	North East	1	0.96	2.88
		2	1.1	3.3
	West	1	ND (0.4)	<1.2
		2	ND (0.4)	<1.2
	South East	1	0.71	2.13
		2	1.2	3.6
Ages <60				
	South	1	1.2	3.6
		2	ND (2)	<6
	Rural	1	1.1	3.3
		2	1.7	5.1
	North East	1	1.3	3.9
		2	1.1	3.3
	West	1	0.86	2.58
		2	ND (0.8)	<2.4
	South East	1	2.1	6.3
		2	1.1	3.3

3. Back-Calculation of TCDD

The elimination rate of TCDD has been evaluated in a variety of studies of persons occupationally exposed (see, for example, Flesch-Janys et al. 1996; Michalek et al. 1996; Michalek and Tripathi 1999; Rohde et al. 1999) with estimates of first-order half-lives in the range of approximately 7 to 9 years. Studies of persons accidentally exposed to high levels of TCDD and related compounds have demonstrated that at elevated exposures, humans eliminate TCDD and related compounds more rapidly than at lower levels (Geusau et al. 2002; Aylward et al. 2005; Kerger et al. 2006; Leung et al. 2007; Ryan et al. 1993). Similar behavior is observed in animal studies and is related to the induction of CYP1A2 enzymes (Diliberto et al. 2001; Diliberto et al 1999).

Two groups have published pharmacokinetic models in an attempt to capture the concentration-dependent elimination behavior of TCDD (Emond et al. 2005; Aylward et al. 2005). In the first model, elimination of TCDD is postulated to occur directly through metabolism of the compound by CYP1A2 (Emond et al. 2005). The dose-response function for induction of CYP1A2 in human liver was modeled based on data from rats. This model predicts impacts of the concentration-dependent elimination rates at levels of TCDD at or near the range of background exposures.

The second model also relies upon induction of CYP1A2 as the mechanism of increased elimination rates in humans, primarily through the observed action of the CYP1A2 protein as a binding protein in the liver for TCDD and a number of related compounds (Diliberto et al. 1999). As the protein is induced, the hepatic concentration of these compounds increases, leading to increased availability for biliary excretion and metabolism, processes responsible for elimination of the compounds. In contrast to the Emond et al. (2005) model, which relied upon rat data for parameterization of CYP1A2 dose-response, Aylward et al. (2005) parameterized the induction dose-response function based on observed data on hepatic sequestration in humans and by fitting serial datasets from 36 individuals from Seveso and individuals from other accidental exposure incidents. The Aylward et al. (2005) model predicts little or no impact of CYP1A2 induction until concentrations in the range of 500 pg/g lipid for TCDD in serum lipid are exceeded.

If the Emond et al. (2005) model is correct, the back-extrapolation of relatively low current TCDD levels could be impacted by the concentration-dependent elimination phenomenon. However, a number of lines of evidence suggest that this concentration-dependent behavior is not important in the lower exposure ranges suggested by the Emond et al. (2005) model. Kerger et al. (2006) and Leung et al. (2007) examined serial sampling datasets in children and adults from Seveso and from the Yusho and Yucheng incidents. For TCDD in the Seveso individuals, Kerger et al. (2006) reported transitions to increased elimination rates at approximately 400 ppt (for children) to 700 ppt (in adults). Leung et al. (2007) found similar transitions for several PCDF compounds in the range of 1,000 to 2,000 ppt. Lambert et al. (2006) examined a marker for CYP1A2 activity in individuals with a wide range of current serum TEQ concentrations and found a concentration-dependent increase in CYP1A2 activity. Benchmark dose modeling of the data suggest a 10% increased rate of elevated CYP1A2 activity at a serum TEQ concentration of approximately 350 ppt TEQ in serum. Finally, the first-order elimination rates for TCDD estimated based on datasets in the range of 10 to approximately 500 ppt TCDD all cluster around values in the 7 to 9 year range, without elimination rates that are significantly affected by concentration (Flesch-Janys et al. 1996; Rohde et al. 1999; Michalek et al. 1996; Michalek and Tripathi 1999).

Thus, application of a first-order elimination model with a half-life in the range of 7 to 9 years to current measured concentrations seems most appropriate for back-extrapolations. If the back-extrapolated concentrations achieved using this approach exceed approximately 300 ppt, application of a concentration-dependent model could be considered if the increased complexity of the effort is warranted.

3.1 Appropriate Procedures for Back-Extrapolation

As discussed above, valid back-extrapolation can only be conducted when current measured levels exceed the range of background serum levels (Steenland et al. 2001a; 2001b). Assuming an individual's measured serum TCDD concentration exceeds the range of background, back-extrapolation can be conducted using a first-order elimination model. Under a first-order elimination assumption, the current measured concentration in an individual, $C_{current}$, can be considered to be the sum of contributions from ongoing

background exposures and residual compound from non-background exposure at an earlier event:

$$C_{current} = C_{background} + C_{residual} \quad (1)$$

And

$$C_{residual} = C_{t0} e^{-k\Delta t} \quad (2)$$

Where

C_{t0} = Concentration due to non-background exposure at the time of last non-background exposure
Δt = time since last non-background exposure in years
k = elimination rate constant in yr^{-1}

The elimination rate constant, k, is related to the half-life estimate, HL, as follows:

$$k = \frac{\ln 2}{HL} \quad (3)$$

The concentration due to non-background exposure at the time of last non-background exposure can then be estimated through rearrangement of these equations as follows:

$$C_{t0} = \left(C_{current} - C_{background}\right) * 2^{\Delta t / HL} \quad (4)$$

Total concentration at time t_0 can then be estimated by adding the contemporary background concentration at time t_0 to the result of equation 4. Several data sets are available and suggest a half-life for elimination of TCDD in the range of approximately 7.2 (Flesch-Janys et al. 1996) to 8.7 (Michalek et al. 1996) years.

3.2 Considerations Relating to Back-Extrapolations

Several previous studies have collected serum TCDD measurements from individuals exposed to dioxins in the workplace in the course of the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and related compounds (for example, Fingerhut et al. 1991, Ott and Zober 1996; Flesch-Janys et al., 1998). In these studies, the measurements were taken years, and in some cases decades, after last occupational exposure. However, the measured TCDD concentrations in many of the workers remained highly elevated above typical background concentrations. Based on such data, several authors have attempted back-calculations of measured concentrations to estimated concentrations at the time of last exposure in such occupationally exposed populations (Ott and Zober, 1996; Steenland et al., 2001a; Aylward et al., 2005a; Flesch-Janys et al. 1998). These back calculations relied upon either simple first-order assumptions regarding the pharmacokinetic behavior of TCDD, or in one case, on a multi-compartment model that

reproduces the observed increased rate of elimination of TCDD at concentrations exceeding approximately 500 ppt (Aylward et al., 2005a).

Such back-extrapolations are inherently highly uncertain due to the substantial inter-individual variability in elimination kinetics of TCDD (Aylward et al. 2005b) and the confounding effect of ongoing (but varying magnitude) low-level dietary exposure (van der Molen et al. 1998). Furthermore, such back-extrapolations rely upon the assumption that earlier levels were, in fact, higher than current measured levels - an assumption that has demonstrated validity for manufacturing cohorts and which may be inferred from measured concentrations in excess of background levels in conjunction with an appropriate occupational history. As discussed above, however, many or most US Vietnam veterans showed no elevation over background in serum TCDD concentrations in samples taken 15 to 30 years following service in Vietnam (CDC 1988), except for individuals involved in direct handling of the herbicides (Michalek et al. 1999). Even within those groups, a substantial proportion showed no elevation in serum TCDD over background levels 15 to 30 years following cessation of exposure.

Once TCDD levels have decreased to within the range of typical background measurements, it is as present not feasible to determine for an individual whether or how much higher the levels were in the past, or whether the individual always had levels within the range of background. As noted by Steenland et al. (2001a), in their evaluation of the US National Institute of Occupational Safety and Health (NIOSH) occupational herbicide manufacturing cohort, "[v]alid back-extrapolation… is not possible once levels have returned to background", pp. 451-452).

The Ranch Hand researchers collaborated with the US NIOSH researchers to examine relationships between diabetes and related outcomes in the US Ranch Hand and NIOSH cohorts (Steenland et al. 2001b). These researchers conducted back-extrapolations to concentration at time of last occupational exposure, an exposure metric used for some of the exposure-response analyses. However, again, these back-extrapolations were conducted only on individuals with measured concentrations in excess of 10 ppt:

Exposure variables analyzed included a dichotomous variable for exposure (exposed *v* non-exposed), as well a continuous variable which was either lipid adjusted TCDD at the time of the examination, or lipid adjusted TCDD back extrapolated to time of last occupational exposure using the estimated half life of TCDD. Back extrapolation was done by assuming a first order elimination model:

$$TCDD_{t1} = TCDD_{to}\exp(-\lambda*\Delta t)$$

where $TCDD_{t1}$ is serum TCDD at the time of last exposure, $TCDD_{to}$ is serum TCDD at the time of the examination, Δt is the time between last exposure and the time of the examination, and λ is 0.0797 based on a half life of 8.7 years. Serum concentrations were measured by the same laboratory for both studies, with the same method. As outlined above exposure-response analyses by back extrapolated TCDD were conducted only among exposed subjects with a TCDD concentration > 10 pg/g lipid at the time of the examination, which excluded all referents from both studies, as well as the "background group" from Ranch Hand, and 33 (13%) of the exposed workers from the NIOSH study.

The rationale for these exclusions was that a meaningful back extrapolated value cannot be calculated for subjects who have returned to background concentrations of TCDD, as it cannot be stated how long they have been at the background concentration. [Steenland et al., 2001b]

Valid back-extrapolation of values within or near background concentration ranges would require detailed information regarding the levels of background exposure to TCDD through diet and other pathways over the time period of back-extrapolation, including knowledge of how such exposures changed over that period. Reliable estimates of the levels of background exposure to TCDD over the period from 1970 to 2000 are not available for the Australian population, although some rough estimates have been made in the US (Lorber 2002; Aylward and Hays 2002). Since the highest measured TCDD concentration in any of the 102 veterans for which data is available is 6.6 pg/g lipid, we conclude that back-extrapolation is unlikely to provide any meaningful results. This conclusion is supported by the fact that the ratio of TCDD to other regularly detected PCDD/PCDF was not elevated and hence the data provide little evidence that indicates exposure. On the other hand uncertainty with the data and the very long period since potential last exposure (> 4 – 5 half-lives) suggests that it is feasible that individuals did receive elevated exposure but the time since the last period of elevated exposure is sufficient so that the current information does not allow back extrapolation.

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