EVALUATION OF SERUM DIOXIN CONCENTRATIONS IN AUSTRALIAN VIETNAM VETERANS WITH DIABETES MELLITUS

Aylward L.L.[1], Toms L.-M.[2], Mueller J.F. [2]

[1] Summit Toxicology, LLP, Falls Church, VA USA
[2] The University of Queensland, National Research Centre for Environmental Toxicology, Brisbane, Australia

Abstract
Agent Orange and other herbicide mixtures sprayed in Vietnam contained TCDD as a specific contaminant. In theory, due to the persistence of TCDD, elevations of TCDD in the blood of veterans compared to the general population could be used as a marker of exposure to Agent Orange, even in samples taken years after the last date of service. We assess the results of de-identified chemical analysis data from blood samples of 102 veterans with diabetes mellitus (DM), obtained by the Australian Commonwealth Department of Veterans' Affairs (DVA) to evaluate whether the data show a selective elevation of TCDD. Based on available data we estimated that the upper bounds of the background range for Australian men aged 45-60 and 60+ would be approximately 4 and 6 pg TCDD/g lipid, respectively. Only 2 of 102 samples from Australian Vietnam veterans exceeded these values, and none of the samples exceeded 7 pg/g lipid. Based on these results, there did not appear to be evidence of selectively elevated exposure to TCDD within this group of veterans. However, the evaluation and interpretation was complicated by the high rate of non-detectable concentrations and the high and variable detection limits.

Introduction
The Australian Department of Veterans Affairs (DVA) collected blood samples between 2000 and 2007 from a non-random sample of 102 Australian Vietnam veterans with DM, aged 40-60 years. The samples were collected as part of the compensation claims process and were analysed for polychlorinated dioxins and furans. DVA asked for an evaluation of the blood sampling results in order to evaluate a) whether the samples showed elevations compared to the Australian general population; and b) if back-extrapolation of the measured concentrations to the time of last service in Vietnam was appropriate. Back extrapolation may be appropriate if the measured concentrations of TCDD can be clearly distinguished from background concentrations; however, once serum TCDD concentrations have returned to the range of background, such back-calculations are invalid (1,2). Strong temporal and age-related trends in dioxin concentrations have been noted in studies in various US and European countries (3,4,5), so identification of an appropriate reference range (age, calendar period) for comparison is important. No comprehensive data are available on the concentrations of dioxins and furans in persons in the Australian general population, although results from the Australian National Dioxins Program (NDP), a statistically representative survey employing pooled blood samples collected in the same time period as the veteran blood samples, have been published (6,7). The results from pooled samples can be used to estimate the central tendency, but not the likely range, of values for persons of various sex and age groups in the Australian general population. This paper describes our evaluation of the sampling data, the methods used to evaluate whether there was a selective elevation of TCDD in the sampled Australian veterans, and the methods used to estimate the likely range of concentrations in the general population in Australia at the time of sampling. Based on these analyses we assess whether back extrapolation is appropriate and the methods that might be used to conduct such extrapolations.

Methods
We examined the analytical data for serum dioxins and furans for issues related to detection limits and other quality assurance/quality control concerns. We conducted several analyses to assess whether the measured TCDD concentrations in the DVA veterans dataset were selectively elevated:

1. We compared the average of the detectable concentrations for each PCDD/PCDF congener in the DVA sample to the average concentrations detected in pooled blood samples from males over age 45 years in the Australian general population from the NDP.
2. We calculated the ratio of TCDD concentrations (potentially associated with Agent Orange exposure) to the concentrations of either 2,3,4,7,8-pentachlorodibenzo-p-furan (4-PeCDF) or 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (123678-HxCDD), neither of which are believed to be associated with exposure to Agent Orange, to evaluate whether these ratios are different in the DVA veteran sample than in the pooled samples from the NDP.
3. We examined two studies reporting dioxin concentrations from relatively large samples of the United States population (8,9) in order to estimate the typical ratio of upper bound (95^{th} or 99^{th} percentile) TCDD concentrations to the median or mean. These ratios were then applied to the pooled sample concentrations from the NDP to estimate a plausible upper bound for the Australian general population for mean aged 45 to 60 years and over age 60 years.

Based on these evaluations, we assessed whether clear elevations in TCDD were evident in the veterans sampled. Based on the outcome of this analysis, we discussed the appropriateness of attempting to back-extrapolate serum TCDD concentrations to the date of last service in Vietnam.

Results

The analytical data were somewhat limited by high and variable detection limits. For example, only 58 of 102 veterans had detectable concentrations of TCDD and the detection limits for those with non-detected levels varied from 0.9 to 9 ppt. Thus, the data must be interpreted cautiously.

Table 1 presents the average concentrations by congener from the pooled blood sample analyses in the NDP for all pools for males aged 45 years and above (6,7) as well as the average concentrations from the Australian veteran sample based only on the concentrations in samples in which the congener was *detected*. Thus, the concentrations reported for the veterans are likely to overestimate the actual average concentration for all 102 veterans. While the average TCDD concentration in the veterans (2.7+/-1.2 pg/g) is higher than the average from the NDP pools (1.1+/-0.3 pg/g >45yr males; 1.3pg/g >60 yr males), the average concentrations for all of the tested congeners are similarly higher than the corresponding NDP pool concentrations. This does not suggest a selective elevation of TCDD in the Australian veterans.

Table 2 presents the distribution of ratios of TCDD, which could be a marker of Agent Orange exposure, to two other congeners not associated with Agent Orange exposure. These ratios are presented both for the Australian veteran group as well as from the US NHANES and Patterson et al. (2004) studies (8,9). The mean or upper bound ratios between TCDD and the other marker congeners in the Australian veteran group were not different from those observed in the two US samples. Thus, this analysis does not suggest a selective elevation of TCDD in the Australian veterans.

Table 3 presents the distributions of serum TCDD concentrations from the two US population studies. The upper bounds of the distributions are approximately 2 to 4 times the mean or median concentrations in the age groups of interest. These data provide one basis for estimating the likely upper bound in the Australian population based on the pooled sampling results from the NDP. If the upper bound in the Australian general population is estimated to be approximately 3 times the central tendency for a given age group (consistent with the results from the US sampling studies), this would suggest cut-points 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. Of individuals in the DVA sample aged 40-60 years, one exceeded 4 pg/g lipid. For those over the age of 60 years, one exceeded 6 pg/g lipid but none exceeded 7 pg/g lipid.

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. 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) (1,2). Based on all of these evaluations, there is no clear evidence of a selective elevation of TCDD concentrations in the sample of 102 Australian veterans, which means that there is no clear evidence of any remaining signal of potential exposure to Agent Orange, and the measured levels do not clearly exceed the estimated range of background TCDD concentrations in the Australian general population. Based on this conclusion, back-extrapolation of measured TCDD concentrations to estimated concentrations at the time of last Vietnam service is not appropriate, because such back-extrapolations cannot be reliably conducted when serum concentrations are not clearly in excess of background levels.

Conclusions

The analytical data were somewhat limited, with relatively high and variable limits of detection. On average, the blood dioxin and furan concentrations in the blood samples from the 102 Australian Vietnam veterans with DM were somewhat higher than the concentrations obtained in pooled blood samples from persons in the general population of the same sex and age ranges (as shown in the Australian NDP), although that conclusion is dependent on the assumptions made regarding non-detectable concentrations. However, the elevations were seen across all dioxin and furan congeners rather than being limited to TCDD, which did not indicate that the elevations were necessarily due to exposure to Agent Orange. The ratios between TCDD and other marker congeners in the veterans were also not different than observed in other population studies, again not indicating any selective elevation of TCDD concentrations. We estimated the likely variation from the central tendency represented in the pooled sampling by examining the variations from the central tendency observed in studies conducted in other countries. Based on this analysis, we estimated that the upper bounds for Australian men aged 45-60 and 60+ years would be approximately 4 and 6 pg TCDD/g lipid, respectively. Based on this evaluation and the estimated age-specific upper bounds of TCDD concentrations, all but two of the individuals in the veteran group fall within the estimated range of typical concentrations in the Australian general population, and none are substantially elevated. Based on this evaluation, back-extrapolation of measured concentrations does not appear to be appropriate for the Veteran sample data.

Acknowledgements

This analysis was funded by the Australian Commonwealth Department of Veterans' Affairs. The analysis and opinions presented here are those of the authors and do not represent the official policy of the Department of Veterans' Affairs. EnTox is a partnership between Queensland Health and The University of Queensland.

References

1. Steenland K., Calvert G., Ketchum N., and Michalek J. *Occup Environ Med* 2001; 58: 641.
2. Steenland K., Deddens J., and Piacitelli L *Am J Epidemiol* 2001; 154: 451.
3. Lorber M. *Sci Tot Environ* 2002; 288: 81.
4. Wittsiepe J., Schrey P., Ewers U., Wilhelm M., and Selenka F. *Environ Res* 2000; 83: 46.
5. Hays S.M., and Aylward L.L. *Regul Toxicol Pharmacol* 2003; 37: 202.
6. Harden F, Müller J, Toms L, Gaus C, Moore M, Päpke O, Ryan J, Hobson P, Symons R, and Horsley K. 2004. Dioxins in the Australian Population: Levels in Blood, National Dioxins Program Technical Report No. 9, Australian Government Department of the Environment and Heritage, Canberra. 119 pp.
7. Harden F.A., Toms L.M., Päpke O., Ryan J.J., and Müller J.F. *Chemosphere* 2007; 67: S318.

8. Patterson D.G., Canady R., Wong L.Y., Lee R., Turner W., Caudill S. *Organohalogen Compounds* 2004; 66: 2878.

9. National Health and Nutrition Examination Survey (2003-2004). Data available at: http://www.cdc.gov/nchs/nhanes.htm.

Table 1. Summary of the concentration (pg/g lipid) of the 13 most commonly detected PCDD/Fs in data obtained from Vietnam Veterans (n=102) and data from Australia's National Dioxins Program (NDP) Serum Blood study (n=2000, 20 pools of 100 individual males older than 45). Also provided is the concentration ratio of the average concentration in the Vietnam Veterans compared to the general Australian population for those individuals with detected concentrations.

	NDP Study (AS) (only Male > 45 years old)			Vietnam Veteran Data (VVD)			
Congener	Mean	SD	Number of Pools (% detected)	Mean	SD	N (% detected)	Conc. Ratio (VVD/AS)
2378-TCDD	1.1	0.3	16 (80%)	2.7	1.2	58 (57%)	2.3
12378-PeCDD	3.0	0.6	20 (100%)	6.6	2.6	79 (77%)	2.2
123478-HxCDD	2.8	1.0	19 (95%)	7.2	3.6	53 (52%)	2.5
123678- HxCDD	19.5	5.3	20 (100%)	35.2	16.4	99 (97%)	1.8
123789- HxCDD	2.9	1.2	20 (100%)	9.8	5.6	72 (71%)	3.4
1234678-HpCDD	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
2378-TCDF	0.4	0.1	4 (20%)	2.1	1.0	14 (14%)	4.8
23478-PeCDF	2.5	0.6	20 (100%)	6.2	2.6	94 (92%)	2.5
123478-HxCDF	2.0	0.5	20 (100%)	4.1	1.8	55 (54%)	2.1
123678-HxCDF	1.8	0.4	20 (100%)	4.4	1.8	63 (62%)	2.5
1234678-HpCDF	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

SD: Standard deviation.

Table 2. Distributions of the mean and 5th to 95th percentiles of the ratio of TCDD:4-PeCDF or TCDD:123678-HxCDD for all individuals or pools with quantified concentrations.

		Ratio, TCDD:4-PeCDF			Ratio, TCDD:123678-HxCDD		
Study/group	Age Group	N	Mean	5th-95th percentile	N	Mean	5th-95th percentile
US NHANES 2003-2004	45-60	133	0.46	0.20-0.83	136	0.09	0.05-0.21
	60+	314	0.49	0.23-0.83	317	0.09	0.04-0.14
Harden et al. 2004[a]	45-60	8 pools	0.50		8 pools	0.06	
	60+	8 pools	0.45		8 pools	0.06	
Australian veteran data	45-60	34	0.46	0.24-0.78	35	0.08	0.04-0.14
	60+	22	0.40	0.22-0.76	24	0.07	0.03-0.21

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

Table 3 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	Ratio, 95th %ile to Mean	Ratio, 95th %ile to Median	Ratio, 99th %ile to Mean	Ratio, 99th %ile to Median
NHANES (2003-2004)										
45-<60	261	56%	2.1 (1.5)	1.8	5.2	6.6	2.5	2.9	3.2	3.7
60+	448	75%	3.8 (3.2)	3.4	6.1	7.9	1.6	1.8	2.1	2.3
Patterson et al. (2004)										
45-<60	160	NR	1.9 (1.6)	1.4	5.0	NR	2.6	3.6	NR	NR
60+	113	NR	3.9 (3.7)	3.2	10.9	NR	2.8	3.4	NR	NR

NR: Not reported