Appendix G

1. CONTROLS OR BASELINE FOR THE PARITUTU DIOXIN STUDY.

Assessment of possible exposures to 2,3,7,8-TCDD originating from the IWD plant depends on comparison of 2,3,7,8-TCDD levels in serum of study subjects with some measure of the expected range of concentrations of 2,3,7,8-TCDD in the serum of the "typical population" not exposed to emissions from IWD, matched as far as possible to the study subjects in terms of at least age and gender, and preferably also taking account of percentage body fat, dietary habits and other relevant lifestyle factors. Possible approaches to establishing "typical population" levels include:

- Use of the 1997 MfE national serum survey, which provides dioxin congener concentrations in the serum of age-, gender-, ethnicity- and regionally-stratified pooled samples taken from non-occupationally exposed people in 1997.
- Collection of new samples from matched controls selected for each subject included in the Paritutu study.
- Use of the 1997 MfE national serum survey, adjusted as far as possible to take account of the likely decrease in dioxin levels between 1997 and 2003/4 .

1.1 MATCHED CONTROL SAMPLES.

The inter-individual variability indicated by the inter-pool variability in the 1997 serum survey (25-130% expressed as a coefficient of variation or standard deviation/mean) means that there is a high probability of quite large differences between the results from study subjects and matched controls, unrelated to any exposure to IWD emissions. Accordingly, an approach based solely on the use of matched controls is unlikely to be able to identify significant increases above typical population levels, because of the uncertainty of the extent to which the particular controls selected accurately represent the typical population.

If the matched control approach were adopted, it would be desirable to match for age, gender, ethnicity, percentage body fat (including body fat history, if possible), dietary habits, exercise, whether breast fed and, for women, numbers of children and the periods for which they were breast feed. On the face of it, these factors are likely to account for a substantial fraction of the inter-individual variability in typical population levels. However, there appears to be no data from extensive sampling of individuals, together with the relevant personal information to determine the extent to which these factors account for inter-individual variability. Accordingly, there maybe a significant element of faith in adopting this approach, particularly given the small numbers of samples that can be included in the study.

It might be considered that controls should be selected from the New Plymouth or Taranaki area. However, given the past history of IWD, including the earliest location being in New Plymouth City, it is not clear how to be certain of avoiding controls who have been subject to some indeterminate level of exposure from IWD. Further, the 1997 MfE serum survey did

not indicate any substantial regional variability and this suggests that the whole New Zealand population, probably excluding New Plymouth and perhaps Taranaki, would be the best population from which to select controls.

1.2 COMPARISON WITH THE 1997 MFE SERUM SURVEY.

Because of the large numbers of samples involved in the 1997 MfE survey, it can be taken to give a good measure of both the levels of dioxins in the typical population for the various strata, and statistical estimates of the inter-individual variability, based on the variability between pools. If dioxin concentrations in the typical population could be taken to be constant between 1997 and 2003/2004 (when the Paritutu samples are to be collected), the survey would clearly provide the best basis for deciding whether particular study subjects have atypically high 2,3,7,8-TCDD concentrations. However, information from a number of sources, including a toxicokinetic model model based on the 1997 survey data, the 1988 and 1998 New Zealand breast milk surveys and dietary intakes studies for the UK in 1982, 1992, 1997 and 2001, all show major decreases in dioxin intakes. Intakes appear to have been decreasing by about 50% every 5 years. The levels of dioxins in any particular individual will not show as fast a decline as the dioxin intakes, but the decline over 6-7 years will nevertheless be substantial. Accordingly, use of the 1997 MfE survey data without adjustment will give excessively high typical population levels for the various strata for 2003/4.

1.3 COMPARISON WITH THE 1997 MFE SERUM SURVEY, ADJUSTED FOR LIKELY DECREASES BETWEEN 1997 AND 2003/4.

The ideal approach to adjusting the MfE survey data to provide the best estimate of typical population levels for 2003/4 would be comparison of the dioxin levels in serum samples collected in 1997 with further samples collected in 2003/4 from the same individuals. Unfortunately, because of the pooling of samples from a number of individuals and because of privacy issues and other practical difficulties it is unlikely that any of the 1997 pools could be reconstructed based on fresh sampling in 2003/4.

Preliminary inquiries indicate that a number of people who participated in a 1994 blood plasma survey would be willing to give blood for further analyses. This option is probably the best available to obtain an estimate of the rate of decrease in dioxin concentrations in blood over recent years. Although it is still not ideal, because there is no way of knowing whether there might have been a more rapid decrease in dietary intakes between 1994 and 1997 than subsequently, it is still very much better than no information and can be expected to give at least a reasonable range of estimates of the likely decrease between 1997 and 2003/4.

The toxicokinetic model already developed provides the best basis for estimating changes in particular serum dioxin levels for age groups from the 1997 survey as they age by 6-7 years, during which there may have been a continuing decline in the levels of dioxin intake. This model combines the New Zealand information about dietary energy intakes, percentages of body fat and international estimates of 2,3,7,8-TCDD half-life to calculate the dioxin content of typical New Zealand food over the past 40 years that accounts for the changes in 2,3,7,8-TCDD concentrations measured in the different age groups of the 1997 survey for men and for women. The substantially independent estimates of the dioxin content of food for both

men and women are in close agreement, indicating that the model is likely to be reasonably reliable.

The model can be used to examine the change in the dioxin content of blood lipid resulting from any selected pattern of dioxin intake over any period. Accordingly, it is possible to examine what decrease in 2,3,7,8-TCDD in blood is likely to result between 1997 and 2003/4, and between 1994 and 2003/4 based on a range of dioxin intake assumptions.

If samples are collected from participants in the 1994 survey, the model can provide estimates of the rates of decrease of 2,3,7,8-TCDD intakes between 1994 and 2003/4. A range of decrease scenarios is likely to be able to account for the expected decrease measured in the blood samples. For example, a smaller decrease occurring shortly after 1994 could have the same effect on 2003/4 blood levels as a larger overall decrease occurring later. This can be used to explore the range of dietary intake decreases that might be reasonable for the 1997-2003/4 period, the implications of which can then be examined using the model again.

In this way, the best estimates of typical population levels of 2,3,7,8-TCDD in 2003/4 will be derived from the 1997 data. These best estimates would then provide the most reliable point of comparison for deciding whether or not blood from any Paritutu subject included in study shows "excess" 2,3,7,8-TCDD concentrations compared with the typical New Zealand population.

Because the age groupings selected 1994 and 1997 surveys do not coincide, direct comparison between the 2,3,7,8-TCDD levels cannot be done. However, there are fairly consistent indications of decreasing 2,3,7,8-TCDD levels in blood lipids between 1994 and 1997. Further analysis of the individual data for the 1994 survey may make it possible to construct different age groupings that would be comparable with the 1997 survey, and this could provide further indication of the decrease between 1994 and 1997, therefore refining the basis for a 1997-2003/4 decrease estimate. However, the small numbers in the 1994 survey mean that this can only be a rough assessment.

2. LABORATORY QUALITY CONTROL

Reports for both of the NZ breast milk studies (1988 and 1998), together with the WHO report for round 4 of the WHO interlaboratory dioxin trials shows that there are very good laboratories and not very good laboratories. Both AgriQuality and Axys are clearly in the very good category. The quality assurance data in the breast milk study reports indicate that we should expect results from these two labs to agree to within about 20% for most congeners. If we had to pick to laboratories at random from the laboratories reporting to WHO in the interlaboratory trials, we could not expect results to agree much better than within a factor of 1.5 or 2, and might get results differing by a factor of more than 5.

This is based on the agreement between DSIR (now AgriQuality) and the WHO mean result for the first round of the WHO interlaboratory trials in 1988 or thereabouts, and similar agreement between AgriQuality and the top German laboratory (Freiburg) in an interlaboratory check on 3 samples in the 1998 breast milk study. Since Axys was one of only three laboratories meeting all of the WHO quality criteria for analysis of dioxins and furans in milk in the 4th round (and the Freiburg laboratory was one of the others), and was

the only laboratory meeting all of the criteria for analysis of blood, it is reasonable to assume that their performance will be similar to that of AgriQuality and the Freiburg lab.

Consideration was given to whether interlaboratory check samples should be analysed before the full set of study samples from Paritutu study subjects, in case these showed a level of interlaboratory variability that would preclude meaningful comparisons between the Paritutu study samples and the adjusted 1997 serum survey data. Because of the standard of laboratories involved, it has been decided that it would be most appropriate to do the interlaboratory quality control samples at the same time as the full study.

The 1998 breast milk study also had a very useful quality assurance check in the form of three milk samples from the 1988 study, which had been frozen for future quality control purposes. It might be worth checking with Simon whether any samples from the MfE serum survey had been retained for similar purposes.

2.1 ANALYTICAL COMPARABILITY FOR THE PARITUTU SAMPLES WITH THE 1997 SURVEY DATA

The comparability of the analytical data from Axys with the data of the 1997 national serum survey, for which the samples were analysed by the Analytical Toxicology Section of the CDC (Atalanta), will be established through the following linkages.

The mean concentrations of 2,3,7,8-TCDD in the various age groups for men and women were closely comparable between the 1997 survey and an earlier 1994 plasma survey conducted by the ESR trace organics laboratory, which subsequently became AgriQuality. The differences between the two sets of results are consistent with expected decreases in population levels of 2,3,7,8-TCDD over a three-year period, and are very much smaller than the inter-individual variability indicated by both the 1994 and 1997 studies. Accordingly, variations in the true 2,3,7,8-TCDD levels in the serum of different individuals are considerably larger than any variability that can be attributed to inter-interlaboratory or intra-laboratory analytical variability.

The excellent performance of the DSIR/ESR/AgriQuality laboratory over the 1988-2000 period is indicated by the very good results obtained in the participation in the 1998 WHO interlaboratory study and the excellent agreement between the AgriQuality laboratory and the Freiburg laboratory for quality control samples from the 1998 breast milk study. This agreement between AgriQuality and Freiburg also indicates good agreement between AgriQuality and Axys, because of the very good agreement between Freiburg and Axys in the 4th round of the WHO interlaboratory study.

It is proposed that the re-sampling of some 1994 survey participants collect sufficient blood to allow samples to be split for analysis by both Axys and AgriQuality. Provided there is good agreement for the samples between Axys and AgriQuality, the agreement between the ESR 1994 survey data and the 1997 survey data shows that the Axys data for the Paritutu subjects can be compared with the 1997 survey data from the perspective of analytical reliability. Accordingly be samples will provide both an interlaboratory for analytical reliability check and a basis for examining decreases in individual serum plasma levels between 1994 and 2003/4.