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THE EVALUATION OF POSSIBLE HEALTH HAZARDS FROM TCDD IN THE ENVIRONMENT

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For several years we have been developing and applying methods for the measurement of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) in the environment (1,2,3). TCDD is present as a contaminant in certain pesticides, including the herbicides 2,4,5-T and silvex (4). Although the concentration of TCDD in these chemicals is very low, the great toxicity of TCDD and its possible accumulation in the environment make it advisable to determine how much TCDD is reaching various human populations and what exposure level might reasonably be considered hazardous to man.

The analysis of animal fat and milk is of particular interest because TCDD concentrates preferentially in lipid components of the body. Our current method for determining TCDD in fat and milk uses neutral extraction, four steps of column chromatography, and analysis by high resolution mass spectrometry (3). Before extraction a known amout of the ^{37}Cl heavy isotopic isomer of TCDD that we synthesized for this purpose is added to each sample to serve as an internal standard. The great specificity and sensitivity of high resolution mass spectrometry make it especially well suited to the measurement of low levels of TCDD. Figures 1 and 2 show examples of TCDD peaks as they are recorded by the mass spectrometer at the two TCDD mass/charge ratios which we routinely use for analysis, m/e = 319.897 and m/e = 321.894. In an individual mass spectrometer run the amount of TCDD is determined by measuring the height of one or the other of these peaks relative to the height of the peak from the internal standard at m/e = 327.885 (not shown).

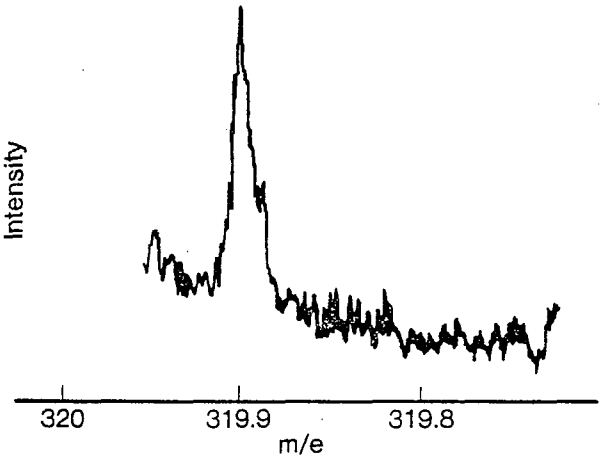

FIGURE 1. Beef fat 1 gram sample 13 PPT TCDD.

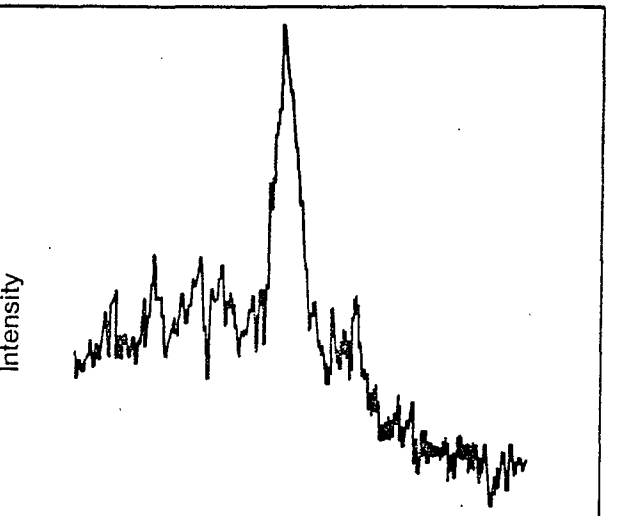

FIGURE 2. Bovine Milk 15 gram sample 0.7 PPT TCDD

Figures 3 and 4 show the results of analyzing samples of beef fat and human milk containing various amounts of added TCDD, submitted to us by the Environmental Protection Agency and the Food and Drug Administration in order to test the sensitivity and accuracy of

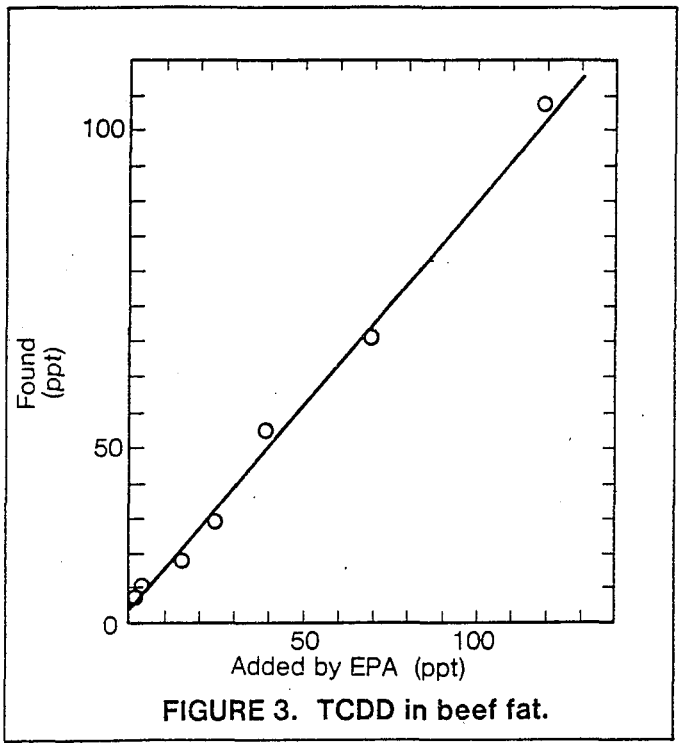

FIGURE 3. TCDD in beef fat.

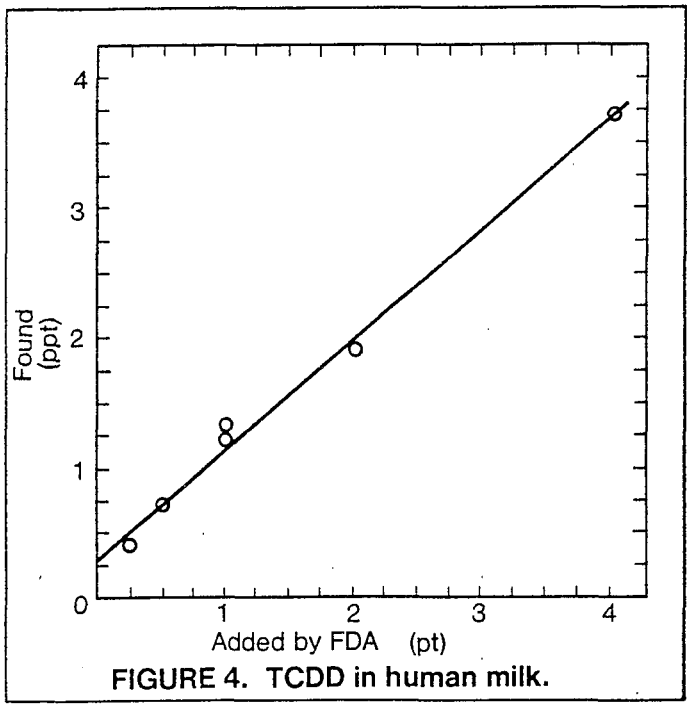

FIGURE 4. TCDD in human milk.

the analytical method. No TCDD above the limit of deduction imposed by background noise in the mass spectrometer was found in control samples without added TCDD. As may be seen, the relation between added and measured TCDD levels is very close to linear over the entire range tested. TCDD was detected when added at levels as low as 2 parts per trillion (ppt) in beef fat and 0.25 ppt in human milk. However, near these limits, the measured amount of TCDD exceeded the amount added by a factor of up to three, an effect we are presently examining. Although analytical methods for TCDD have improved enormously over the last several years, further refinements are underway to permit accurate measurements at even lower concentrations and to provide improved discrimination among the positional isomers of TCDD, some of which may be present in the environment in addition to the 2,3,7,8 isomer (5,6).

As part of the initial phase of an effort by EPA to monitor TCDD, analyses have been done by a number of laboratories on fat from cattle grazed on 2,4,5-T–treated rangeland in Kansas, Missouri, Oklahoma, and Texas and from cattle grazed on untreated land. We received for analysis by our current method 14 samples from the 2,4,5-T group and one control. The samples were selected to include several which had been reported to contain TCDD by other laboratories.

We found TCDD in 11 of the samples from treated rangeland but none in the control or in beef fat samples from a Cambridge, Massachusetts, market. The four samples with the highest levels were found to have 70, 24, 20, and 12 ppt, respectively. The overall results of our analyses and those of others participating in the study were summarized by EPA in June 1976, as follows:

> Of the fat samples (85) analyzed, one shows a positive TCDD level at 60 ppt; two samples appear to have TCDD levels at 20 ppt; five may have TCDD levels which range from 5–10 ppt. While several laboratories detetced levels (5–10 ppt) in this range, the values reported were very near the sample limits of detection. There exists a great deal of uncertainty of the analytical procedure below 10 ppt.

This interim summary needs a little clarification. Actually, the number of beef fat samples was 89, of which 68 were from the 2,4,5-T group and 21 were controls from unsprayed land. No consistent finding of TCDD was reported for the controls, of which 17 were analyzed at a sensitivity of 10 ppt or better, 10 of them by more than one laboratory. Only 25 samples from the 2,4,5-T group were analyzed at a sensitivity of 10 ppt or better by more than one laboratory. Among these 25 there were nine samples for which two or more laboratories reported positive TCDD levels, one sample at *ca.* 65 ppt. two at *ca.* 20 ppt, and six in the range *ca.* 5–20 ppt. This ignores

positive results obtained by low resolution mass spectrometry since they are unreliable. If one employs somewhat less stringent criteria for including samples in the tally, while still excluding low resolution positives, there are several more samples for which TCDD levels of *ca.* 5–30 ppt were reported plus numerous ones in which TCDD was not detected. Since June 1976 EPA has accumulated more data, and it is to be hoped that this and the data on which the 1976 statement were based will be released before much longer.

There appears to be a significant association between the use of 2,4,5-T and the positive TCDD analyses of beef fat. This is not altogether unexpected at the application levels used, *ca.* 1 lb 2,4,5-T/acre with *ca.* one head of cattle per 2 sprayed acres and assuming there was about 0.1 part per million (ppm) TCDD in the 2,4,5-T. Under these conditions the accumulation of a few ppt of TCDD in beef fat would correspond to only a small percentage of the amount applied per head. Nevertheless, it is possible that at least some of the TCDD came from now-discontinued industrial operations in Missouri known to have released TCDD into the environment. More analyses of samples from carefully chosen locations may be needed to settle this point.

Meanwhile, we have taken a different and possibly more direct approach to estimating human exposure to TCDD, through the analysis of human milk. This can provide a measure of the level of TCDD intake of the individual. In a preliminary study we analyzed milk samples from 18 women living in areas where 2,4,5-T is used on rangeland or in forestry and six women from the Boston area. We found four positive samples (with about 1 ppt each) in the former group and none in the latter. This possible association with the use of 2,4,5-T does not involve a large enough number of samples to be statistically significant. Nevertheless, it has led us in collaboration with the National Institute of Environmental Health Sciences to initiate a somewhat larger study, which includes blanks and calibration samples interspersed among the samples from 2,4,5-T areas. Analyses for TCDD in mother's milk on a still large scale are being undertaken by the EPA using samples from women living near sprayed forests in the Pacific Northwest.

As estimates become available for the level of human exposure to TCDD, more accurate information will be needed regarding the level of chronic exposure which may be toxic. The EPA has attempted to estimate levels below which there is unlikely to be any detrimental effect in man, using laboratory data from long-term feeding of TCDD to rats. This use of long-term exposure data is important because there are indications that the toxic effects of TCDD may be extraordinarily cumulative (7). However, the rat is not a very appropriate species for making extrapolations to man. It is relatively insensitive to the lethal effect of TCDD when compared with other species such as the guinea pig and, more importantly, the rhesus monkey.

It is already clear from a 9-month feeding experiment that the lethal level for chronic TCDD exposure in monkeys is less than 500 ppt in the diet, possibly much less (8). If TCDD toxicity were completely cumulative in the monkey, the lethal chronic dietary level could be about 20 ppt. Toxicity of a different nature at even lower levels is suggested by a report that TCDD can be carcinogenic to rats at dietary levels as low as 5 ppt (9). Although there is no evidence that anyone in the U.S. is receiving this much TCDD on a steady basis, it is customary to set the permissible level of human exposure to toxic substances very much below the levels found to be lethal or carcinogenic to laboratory animals. Thus, considering the range of uncertainty in both the level of human exposure and the level which might be toxic, it cannot yet be said whether or not current environmental exposure to TCDD poses a serious, widespread hazard. However, progress in analytical methodology and in understanding the toxicology of TCDD is continuing and, if efficiently exploited, should provide a greatly improved perspective on the TCDD problem before much longer.

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