Dioxin Concentrations in the Blood of Individuals Resident on Farms Near Bolsover, U.K.

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Introduction

Following the discovery of high levels of certain dioxins in cows milk from three farms situated close to Bolsover, Derbyshire [1,2], officials offered to have analysis undertaken of blood samples from any individuals who had resided on these farms and had a history of consuming their own produce. A number of individuals, including residents from each of the three farms, subsequently asked for analysis to be undertaken and provided samples.

Experimental

All samples, which consisted of plasma separated from a unit of blood, were taken at a local hospital and transported to the laboratory in a frozen state contained in standard flexible plastic transfer packs.

To provide controls a plasma sample separated from a unit of blood from a random donor (collected into a transfer pack identical to those used for the above samples) (Sample 11) and a sample of the supernatant left after cryoprecipitation of pooled plasma from a large multi-donor pool (Sample 12) were analysed. Preservative solution from unused transfer packs was analysed as a field blank.

Internal standard solution containing 9 different $^{13}C_{12}$ PCDDs and PCDFs was added to plasma (150 g) and left to equilibrate for 1 hour. Ammonium hydroxide (20 ml) and ethanol (50ml) were added followed by extraction with diethyl ether/pentane (1:1; 150 ml) The aqueous portion was re-extracted with further portions of diethyl ether/pentane (100ml) until a colourless extract was obtained. The combined extract was reduced to dryness and the mass of extracted lipid determined gravimetrically.

PCDDs and PCDFs were isolated by the method of Smith *et al* [3] using an automated apparatus (Fluid Management Systems) [4]. The collected fraction was solvent exchanged into hexane, passed through a disposable mini-column containing H_2SO_4 on silica gel, silica gel and KOH on silica gel, and then chromatographed on a disposable Florisil column eluting PCDDs and PCDFs with dichloromethane (30 ml). The final extract was concentrated just to dryness. Extracts were re-dissolved in nonane (25 µl) containing $^{13}C_{12}$ 1,2,3,7,8-PeCDF and 1,2,3,4,7,8,9-HpCDF.

GC/MS was performed on a VG AutoSpec instrument fitted with a Carlo Erba 5300 Mega Gas Chromatograph and CTC A200S autosampler. The GC was fitted with either a Restek RTX5 or J&W DB5 fused silica capillary column (60 m; 0.25 mm i.d; 0.10 µm film thickness). Splitless injections of 1.5 µL were made at an injector temperature of 280°C and with a splitless period of 99 s. The column oven temperature programme consisted of a 3 min isothermal period at 100°C followed by heating at 25°C/min to 200°C and then at 3°C/min to 300°C with a final hold of 5 min. The GC/MS interface was set to 250°C. Electron ionization was used with an ion source temperature of 250°C, an electron energy of approximately 40 eV and a trap current of 800 µA. The mass spectrometer was operated at a resolution of at least 7000 (based on peak width at 5% of peak height). Selected ion monitoring was employed using the two most intense ions from the molecular ion cluster for each homologue.

All analytical data were assessed for compliance with acceptance criteria [5]. Each autosampled GC/MS run was preceded by analysis of a standard reference solution used to check system performance and calibration validity. Hardcopies of all integrated chromatograms were scrutinised to assess chromatographic peak shape, resolution and signal-to-noise; lock-mass check traces were examined for evidence of ionisation suppression; and isotope ratios were compared with theoretical abundances. Where GC/MS data were unsatisfactory the extract was re-injected. Extracts were prepared in batches of 5 (or less) which included at least one full method blank.

These methods have been validated by participation in WHO/EURO co-ordinated interlaboratory quality control studies [6].

Results

The two field blank samples were completely free of analytically significant responses.

Table 1 gives the concentrations of PCDDs and PCDFs calculated on a fat basis. As described above, the fat content of the plasma was obtained by weighing the residue after evaporation of solvent from the initial extraction. It should be noted that this determination was incidental to the determination of PCDDs and PCDFs and was of uncharacterised accuracy and precision. The results should be treated with some caution although a single duplicate pair gave reasonable agreement.

Discussion

There is no established UK reference range for PCDDs and PCDFs in blood plasma; in fact there is no readily available UK data for comparison. There is, however, an extensive literature from other countries. The data obtained by Päpke on background and occupationally exposed workers in Germany [7] provide reasonable and useful comparisons.

The distribution of congeners in the samples from Bolsover is dominated by 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD to a far greater extent than would normally be expected.

For 2,3,7,8-TCDD the background range reported by Päpke was 0.6 to 9.1 ng/kg fat with a mean of 3.6. Levels of 1,2,3,7,8-PeCDD were within the range 2.1 to 39 ng/kg fat with a mean of 13.8, and levels of 1,2,3,6,7,8-HxCDD were in the range 15.0 to 124 with a mean of 54.6. In TEQ terms the sum of all congeners was in the range 11.6 to 93.5 with a mean of 40.8 ng TEQ/kg fat.

Comparison with Table 1 reveals that the control samples (Samples 11 and 12) are contained within the background range reported by Päpke. The results for Subject 10 are higher than the controls, especially for 2,3,7,8-TCDD, but within or only just above the German background range. The results for Subjects 2 and 7 are at the upper end of the German background range in terms of TEQ but 2,3,7,8-TCDD makes an unusually large contribution and clearly exceeds the German background range. The remaining samples are, in our opinion, unmistakably higher than the normal background and are within the range found by Päpke for occupationally exposed workers.

References

1. N. Harrison and J.R. Startin, Dioxins in milk: A case study on localised contamination, Submitted for presentation at Dioxin 94, Kyoto, Japan.
2. J.R Startin, C. Wright, M. Kelly, M. Rose and N. Harrison, Levels of PCDD and PCDF congeners in milk from farms near Bolsover, UK, Submitted for presentation at Dioxin 94, Kyoto, Japan.
3. L.M. Smith, D.L. Stalling and J.L. Johnson, Anal. Chem., **56**, 1830 (1984).
4. W.E. Turner, S.G. Isaacs and D.G. Patterson Jr., Chemosphere, **25**, 805 (1992).
5. P.F. Ambidge, E.A. Cox, C.S. Creaser, M. Greenberg, M.G. de Gem, J. Gilbert, P.W. Jones, M.G. Kibblewhite, J. Levey, S.G. Lisseter, T.J. Meredith, L. Smith, P. Smith, J.R. Startin, I. Stenhouse and M. Whitworth, Chemosphere, **21**, 999 (1990).
6. E J Yrjänheikki, Environment and Health in Europe 37; Levels of PCBs, PCDDs and PCDFs in human milk and blood; Second round of quality control studies; WHO, Copenhagen (1991).
7. O. Päpke, M. Ball and A. Lis; Various PCDD/PCDF patterns in human blood resulting from different occupational exposures; Presented at 11th International Symposium on Chlorinated Dioxins and Related Compounds, Research Triangle Park, North Carolina, USA, 23-27 September 1991.

Table 1. Concentration of PCDDs and PCDFs found in plasma (ng/kg) calculated on a fat basis.

Subject	1	2	3	4	5	6	7	8	9	10	11	12
Farm	B	B	B	B	B	A	A	A	C	C	-	-
Sex	M	F	F	M	M	F	F	F	M	F	-	-
Age	45	20	48	47	23	53	22	69	35	34	-	-
2,3,7,8-TCDD	131	58	189	129	202	113	43	148	78	14	3	2
1,2,3,7,8-PeCDD	75	25	57	57	88	43	22	47	40	19	8	5
1,2,3,4,7,8-HxCDD	19	8	17	12	17	28	12	32	20	16	10	8
1,2,3,6,7,8-HxCDD	106	85	92	150	178	116	70	130	106	55	44	26
1,2,3,7,8,9-HxCDD	21	25	22	38	42	49	32	47	35	25	10	4
1,2,3,4,6,7,8-HpCDD	141	153	127	103	91	253	177	215	242	243	223	76
OCDD	2270	2150	1750	1820	1310	3910	3350	3330	1150	1040	1850	1330
2,3,7,8-TCDF	1	3	2	1	2	2	2	1	5	<2	2	<1
1,2,3,7,8-PeCDF	<1	<2	<2	<1	<2	1	<1	<1	<3	<1	1	<1
2,3,4,7,8-PeCDF	33	18	32	28	39	46	21	55	31	21	12	9
1,2,3,4,7,8-HxCDF	<1	8	12	9	14	24	11	21	10	6	8	5
1,2,3,6,7,8-HxCDF	12	10	13	12	19	21	13	19	11	7	7	4
1,2,3,7,8,9-HxCDF	<2	<2	<2	<1	<2	2	<2	<2	2	<1	<1	<1
2,3,4,6,7,8-HxCDF	5	8	5	5	6	10	7	8	5	4	3	1
1,2,3,4,6,7,8-HpCDF	18	23	20	31	31	29	18	19	13	9	24	10
1,2,3,4,7,8,9-HpCDF	<5	<2	<2	<1	2	3	<2	<2	<2	<1	2	<1
OCDF	8	8	8	4	8	5	4	3	<4	<2	5	2
Summed TEQ	205	95	247	197	291	190	85	231	137	49	26	16
Fat (%)	0.69	0.4	0.61	0.66	0.64	0.49	0.29	0.42	0.64	0.87	0.63	0.63