Bull. Environ. Contam. Toxicol. (1988) 40:604–611

Archives of Environmental Contamination and Toxicology

Distribution of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in Human Whole Blood and its Association with, and Extractability from, Lipoproteins

L. Omar Henderson and Donald G. Patterson, Jr.

Division of Environmental Health Laboratory Services, Center for Environmental Health and Injury Control, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333

Many scientists consider 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to be the most potentially lethal man-made toxicant. It is primarily a byproduct and contaminant in municipal incinerators and automobile exhausts and in the production of phenoxy acid herbacides (Kimbrough 1980), especially herbicide Orange, (albeit in extremely small quantities--0.1 to 47 ug/g). The extraordinary physiochemical properties of the compound stem, in part, from the extreme hydrophobicity (Chlou et al. 1977). It is introduced into the environment through industrial accidents and human distribution through negligence or error.

Little is known about the specific mechanism of human toxicity or the long term health effects of TCDD exposure. Acute effects such as chloracne may appear and disappear before more long-term effects mainifest themselves, such as possible immune system suppression (Hoffman 1986). The distribution of TCDD in the human body has not been documented. Due to its lipophilic nature, however, it has been quantitated from extracts of adipose tissue, breast milk, and, more recently, in serum (Ryan et al. 1986; Patterson et al. 1987). The transport and eventual equilibration of most xenobiotic substances proceeds from the blood into tissues. Lipoproteins, the combination of protein and lipid, are efficient vehicles for various lipophilic xenobiotic substances in the blood (Maliwal et al. 1982).

In vitro experiments (Roboz et al. 1985) have demonstrated that lipoproteins are carriers of polyhalogenated biphenyl compounds, which are added directly to serum, or to lipoprotein solutions. Investigators have studied the partitioning of TCDD among lipoprotein species (Marinovich 1983), but the potential associations with other serum proteins and red blood cells have not been thoroughly documented. In developing and documenting a method for quantifying TCDD in serum samples, we needed to determine its distribution and availability for extraction in the blood. We sought to determine the partitioning of TCDD (when added in vitro to whole blood), among the cellular, serum protein, and lipoprotein components.

Send reprint requests to Dr. L. Omar Henderson at the above address.

MATERIALS AND METHODS

^{3}H-2,3,7,8-tetrachlorodibenzo-p-dioxin (^{3}H-TCDD) was obtained commercially (N.E. Nuclear-Dupont, Boston MA). Its purity and isotopic labeling (99%) were certified by the manufacturer. Figure 1 outlines the experimental design. Briefly, whole blood collected in ethylenediaminetetraacetic acid (1-mg/mL) from three normolipemic donors was split into two 10-mL aliquots. To the first aliquot, a standard amount (45-ug, 65,000 counts per minute {CPM}) of ^{3}H-TCDD was added in 10-uL of hexane and mixed by magnetic stirring for one hour at room temperature. Previous data indicate an almost immediate uptake of TCDD into the plasma protein and lipoprotein fractions, which remains constant (Henderson, unpublished). A similar aliquot of ^{3}H-TCDD was added to the plasma fraction after cellular material was removed by low speed centrifugation. Plasma and red blood cells were then recombined and thoroughly mixed. Both aliquots of blood were centrifuged at low speed to separate the plasma and red blood cell-containing fractions. Red blood cells were resuspended in isotonic saline solution (0.15 M) and recentrifuged. The saline wash was added to the plasma fraction. Aliquots of red blood cells, plasma, and lipoprotein-containing solution (after ultracentrifugation at 1.21-g/mL, Havel et al. 1955) were counted in a scintillation counter (Beckman Instruments, Palo Alto, CA). The lipoprotein-free supernatant (after precipitation of lipoproteins with microparticulate silica, Agnese et al. 1983) was also counted in a scintillation counter. Quench correction was calculated after recounting the samples with added ^{3}H toluene (10,000 CPM, N.E. Nuclear-Dupont, Boston, MA).

Distribution of ^{3}H-TCDD among lipoprotein fractions was determined in ultracentrifugally isolated lipoprotein fractions corresponding to very low density lipoprotein ({VLDL} $d \leq 1.006$-g/mL), low density lipoprotein ({LDL} d = 1.006-1.063-g/mL), high density lipoprotein ({HDL} d = 1.063-1.21-g/mL) and in the >1.21-g/mL fractions isolated from the sera of three normolipemic donors.

To determine the distribution of ^{3}H-TCDD among non-lipoprotein plasma protein components, we treated an aliquot of the 1.21 g/mL infranatant fraction, produced by ultracentrifugation, with 6.0 M guanidine hydrochloride (GuHCl). Then it was sieved on a Sephadex A 0.5M (Pharmacia, Upsula, Sweden) column (1.2 X 100 cm) eluted with 6.0 M GuHCl buffer, pH 8.6. Radioactive ^{3}H-TCDD was quantified in the column fractions, and elution profiles were compared with authentic molecular weight calibration materials which had been run through the same column previously.

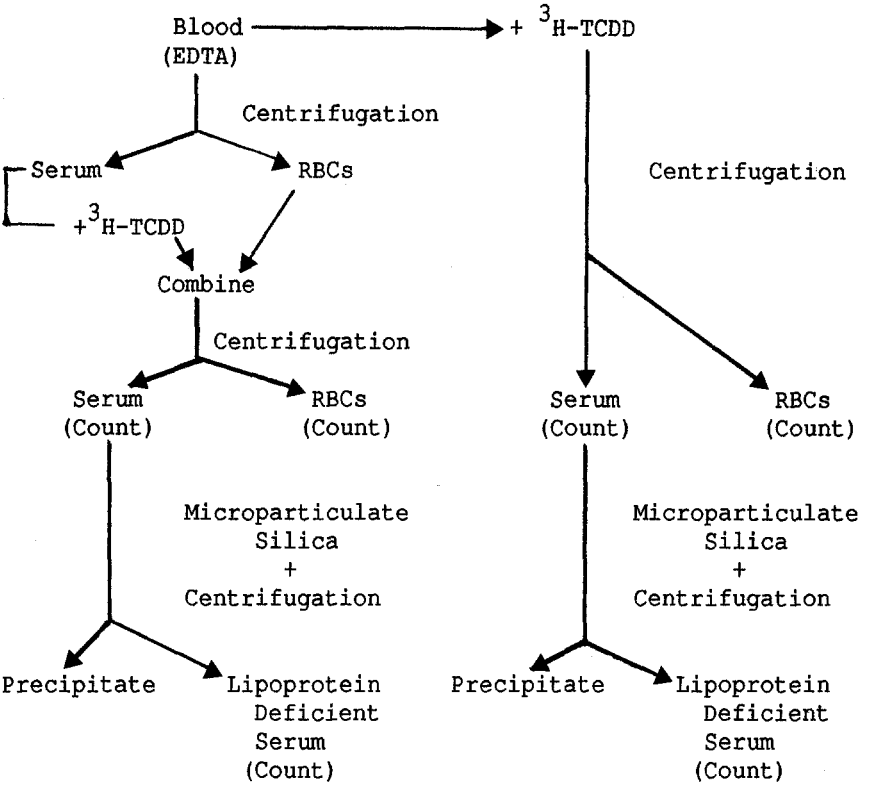

Figure 1 Flow chart of experimental addition of ^{3}H-TCDD to blood and separation into serum, cellular and lipoprotein fractions

In order to estimate extraction precision, eight 10-g samples of an "unspiked" human serum pool or serum pools (prepared by combining multiple donors) were treated with microparticulate silica. Microparticulate silica precipitates were separated from serum by low speed centrifugation. Absolute ethyl alcohol (50 mL) was added, and the suspended precipitate was stirred for 1 hour. The precipitate was further extracted with three 100-mL washes of hexane and intermediate low speed centrifugation to separate the solvent and precipitate. Further treatment of the hexane extracts and quantification of TCDD by high resolution mass spectrometry has been described (Patterson et al. 1986 and 1987).

RESULTS AND DISCUSSION

In three experiments radioactive ^{3}H-TCDD, added to whole blood or to plasma that was recombined with red blood cells, was totally recovered in the plasma fraction after we removed the red blood cells. Recoveries from whole blood averaged 105.3% $\pm$ 4.0% SD and recoveries from reconstituted blood averaged 100.7% $\pm$ 1.1% SD.

In these experiments we made no attempt to separate and quantify radioactivity in the buffy coat containing the immunocytes. In other experiments, however, we determined that the white blood cellular population contained less than 5% of ^{3}H-TCDD added to whole blood. Because tritium was quenched by hemoglobin and the serum itself, the absolute analytical measurement error was 5%.

Table 1 presents statistical data for a 2,3,7,8-TCDD spiked pool (10g). The mean percent recovery of the internal standard was 63%. The limit of detection of the method (Patterson et al. 1987) in environmental samples was about 1 part per trillion.

Table 1 Statistical data for the 2,3,7,8-tetrachlorodibenzo-p-dioxin spiked human serum quality control pool

Concentration of 2,3,7,8-TCDD[a]	1.74
Standard deviation	0.30
Coefficient of variation	17.5%
number of samples, (size in g)	8(10)
99% control limits, upper	2.53
99% control limits, lower	0.96
95% control limits, upper	2.34
95% control limits, lower	1.15
Mean percent recovery of internal standard	63
number of samples, (range of recoveries)	6(43%-95%)

a = parts per trillion

Recoveries of TCDD from four human serum pools (200-g), each measured two to four times (Table 2), averaged 65% recovery of expected levels of the internal $^{13}C_{12}$-TCDD standard with percent coefficient of variation (CV%) for the measurement of TCDD ranging between 1% and 33%.

Table 2 Summary of results from unspiked human serum pools (200-g samples)

Human serum pool	Mean conc[a]	Standard deviation	CV%	n	Mean recovery
C	21.7	4.1	19	3	78%
D	18.8	0.3	1	2	56%
G	30.1	9.8	33	4	59%
H	38.2	8.7	3	2	66%

a = parts per quadrillion

After quench correction, recoveries of ^{3}H-TCDD in ultracentrifugally prepared lipoprotein fractions averaged between 84% and 89% (n = 3), respectively, from whole blood and reconstituted blood. Lipoprotein deficient fractions (>1.21 g/mL) contained between 11% and 16% of the added tracer from the two blood preparations. We recovered about 4% more tracer from the reconstituted preparation.

The distribution of ^{3}H-TCDD among lipoprotein fractions from three fasting, normolipemic donors indicated a greater percentage of tracer associated with LDL (55.3% $\pm$ 9.03% SD) than with VLDL (17.4% $\pm$ 9.07% SD) or HDL (27.3% $\pm$ 10.08% SD). The large SDs in the distribution of ^{3}H-TCDD among lipoprotein fractions were due partially to problems with multiple centrifugation, and partially to our method of handling during transfer, which decreased the analytical recovery (average 79% of the lipoprotein associated TCDD).

Column chromatography in protein denaturing buffer (6.0 M GuHCl) of the lipoprotein-deficient serum (Figure 2) indicated that the majority of ^{3}H-TCDD found in this fraction, about 15% of the total radioactivity added to whole blood, is found in association with proteins in the molecular weight range of <70,000 daltons, with very little associated with proteins <20,000 daltons or >200,000 daltons. The major radioactive peak corresponds to the elution profile of human serum albumin. Very little radioactivity was recovered in molecular weight ranges of the apolipoproteins associated with either LDL (apolipoprotein B, >250,000 daltons) or HDL (apolipoprotein A-I, 27,000 daltons).

TCDD is one of 22 tetra isomers. The entire class of dioxins have very low water solubility and, therefore, a high partition coefficient between water and organic solvent (e.g., hexane). This hydrophobic quality of TCDD would predict accumulation in a nonpolar mileu in an aqueous environment such as blood (Brown et al. 1984).

The main classses of compounds effecting transport of hydrophobic substances in the blood are: (1) amphipathic proteins capable of both hydrophilic and hydrophobic interactions (e.g., albumin); and, (2) lipoprotein molecules that present charged amino acids of proteins and polar head groups of phospholipids to the aqueous phase while sheltering hydrophobic compounds such as cholesterol esters, triglycerides, and other hydrophobic compounds in the core.

The distribution of polychlorinated biphenyls (PCBs) and pesticides in whole blood and reconstituted blood fractions has been studied (Morgan *et al.* 1972). PCBs distribute preferentially to the lipoprotein fraction with small amounts associating with other proteins--primarily albumin, globulins, and cellular components of blood. When we added ^{3}H-TCDD to either whole blood or serum reconstituted with red blood cells, it associated entirely with the red cell free phase and substantially (>80%) with lipoproteins.

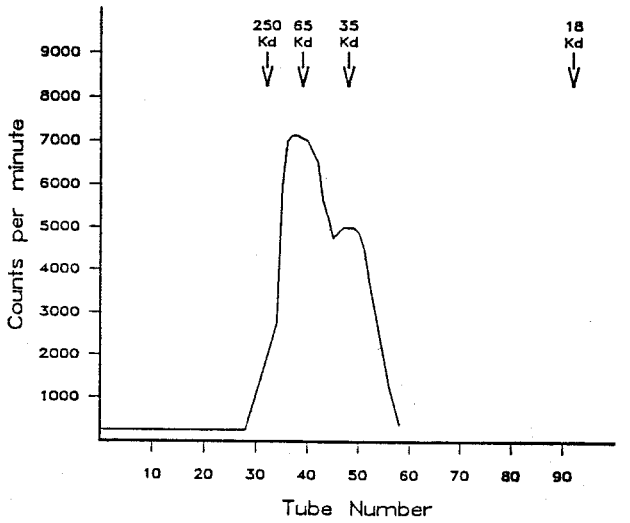

Figure 2 Chromatogram of ^{3}H-TCDD associated with serum proteins

Chromatogram of elution pattern on Sephadex A 0.5M with 6.0M guanidine HCl. ^{3}H-TCDD (~15% of 200,000 CPM added to whole blood) associated with plasma proteins. Molecular weight markers at arrows: 250 Kilo daltons = human serum globulin; 65 Kilo daltons = human serum albumin; 35 Kilo daltons = pepsin; 18 Kilo daltons = beta lactoglobulin.

In aqueous solutions, TCDD and other nonpolar compounds also associate with substances such as silica and soil (McConnell et al. 1984). Part of the potential losses in recovery in the present study may be due to adsorption to glass or the microparticulate silica used to remove lipoproteins. Our data showed that an average of 64% of the $^{13}C_{12}$-TCDD was detected in samples after extraction of lipoproteins with microparticulate silica, sample cleanup, and measurement. By using teflon labware in the later phases of extraction experiments we got higher yields and decreased variability in recovery, as well as 75%-80% recoveries of $^{13}C_{12}$-TCDD. These results are comparable to those obtained with other reported extraction procedures (Patterson et al. 1987).

Measuring the distribution of TCDD in human tissue is an important current topic. Assessment of health effects in occupational and accidental exposures to TCDD will be more accurate if body burden measurements are obtained. Depot adipose tissue is a rich and consistant source of accumulated TCDD, and estimates of body burden have been reported on concentrations of TCDD in this tissue (Patterson et al. 1986). Adipose tissue is a dynamic tissue that exchanges substances freely with blood. A less invasive measurement of TCDD (extracted from blood) closely approximates levels measured in adipose tissue (Patterson et al. 1987)--when lipid components of blood are compensated for in the calculations. It follows that the major transport vehicle, lipoproteins, are an

important source material from which to obtain and measure TCDD from the blood. Indeed, the levels, distribution, and metabolism of lipoprotein species may modulate the biological effects of TCDD (Marinovich et al. 1983).

Measurements of ^{3}H-TCDD recovered from the various lipoprotein fractions (VLDL, LDL, HDL) were similar to those previously reported (Marinovich et al. 1983). Large analytical losses (averaging 21%) during the three-step, 72-hour isolation procedure, however, prevented us from accurately correlating the distributions with subjects' levels of particular lipoprotein species. Individual variations in the amounts of each lipoprotein class may alter the distribution of TCDD among lipoproteins in a given subject. Although the bulk of TCDD is transported in the LDL fraction, evidence suggests that triglyceride-rich VLDL may have a stronger affinity for TCDD than does LDL (Marinovich et al. 1983). Measurements on the association of TCDD with chylomicrons have not been reported. This transient class of lipoproteins may be an important key to the transport of TCDD delivered to the alimentary tract. After absorption, lipids and lipophilic substances, (including fat-soluble vitamins) are repackaged in the intestine into chylomicron particles that enter the blood stream.

Though we could not measure the total amount of ^{3}H-TCDD associated with red blood cells, up to 5% may be associated with the immunonocyte population, which contains the functional humoral immunological components of the body. TCDD's effects on immunological status are not yet clear. Cellular uptake of TCDD by other human cells (in vitro via lipoproteins), however, has been documented (Shireman et al. 1986). Cellular lipoprotein receptors, especially LDL receptors, may be important in the uptake and subsequent cellular effects of TCDD. Delivery of TCDD to cells by lipoproteins may have important physiological consequences, because the partitioning of TCDD in blood favors the lipoprotein vehicle.

Acknowledgments We would like show our appreciation to L. Hampton for technical assistance and Dr. L. Alexander for analytical measurements. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

REFERENCES

Agnese S, Spierto F, Hannon WH (1983) Evaluation of four reagents for delipidation of serum. Clin Biochem 16:98-100

Becker MM, Gamblee W (1982) Determination of the binding of 2,4,5,2',4',5'-hexachlorobiphenyl by low density lipoprotein and bovine serum albumin. J Toxicol Environ Health 9:225-234

Chlou CT, Freed VH, Schmedding DW, Kohnert RL (1977) Partition coefficient and bioaccumulation of selected organic chemicals. Environ Sci Tech 11 (5):475-478
Havel RH, Eder H, Bragdon J (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 34:1334-1353
Hoffman RE, Stehr-Green P, Webb, K (1986) Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. JAMA 255(15):2031-2038
Kimbrough RD (1980) Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products: vol 4. Elsevier/North Holland Biomedical Press, New York
Malival BP, Guthrie F (1982) In vitro uptake and transfer of chlorinated hydrocarbons among human lipoproteins. J Lipid Res 23:474-479
Marinovich M, Sirtori C, Galli C, Paoletti R (1983) The binding of 2,3,7,8-tetrachlorodibenzodioxin to plasma lipoproteins may delay toxicity in experimental hyperlipidemia. Chem Biol Interactions 45:393-399
McConnell EE, Lucier G, Rumbaugh R (1984) Dioxin in soil: bioavailability after ingestion by rats and guinea pigs. Science 223:1077-1079
Patterson DG Jr, Hampton L, Lapeza C Jr, Belser W, Green V, Alexander L, Needham L (1987) High-resolution gas chromatography/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodebenzo-p-dioxin. Anal Chem 59(15):2000-2005
Patterson DG Jr, Holler J, Lapeza C, Alexander L, Groce D, O'Connor R, Smith SJ, Liddle J, Needham L (1986) High-resolution gas resolution mass spectrometric analysis of human adipose tissue for 2,3,7,8 tetrachlorodibenzo-p-dioxin. Anal Chem 58(4):705-13
Patterson DG Jr, Needham LS, Pirkle JL, Roberts DW, Bagby J, Garrett WA, Andrews JS, Falk H, Sampson EJ, Houck VN (1988) Correlation between serum and adipose levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 50 persons from Missouri. Arch Environ Contam and Toxicol 17(2):139-143
Roboz J, Greaves J, McCamish M, Holland J, Bekesi G (1985) An in vitro model for the binding of polybrominated biphenyls in environmentally contaminated blood. Arch Environ Toxicol 14:137-142
Ryan JJ (1986) Distribution of chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans in human tissue from the general population. In: Rappe C, Choudharg G, Keith, LH (eds) Chlorinated Dioxins and Dibenzofurans in Perspective. Lewis, Michigan, p 3
Shireman RB, Ci Wei C (1986) Uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin from plasma lipoproteins by cultured human fibroblasts. Chem Biol Interactions 58:1-12

Received November 17, 1987; accepted January 20, 1988.