Lipid Extraction in Human Blood and Concentrations of Dioxin-like Compounds

John J. Ryan and Pat Mills
Health Canada, Bureau of Chemical Safety, Tunney's Pasture, Ross Ave, Ottawa K1A 0L2, CANADA

Introduction

In analytical chemistry, the term concentration is usually defined as the amount of a substance per unit of sample. More simply this number is a fraction consisting of a numerator containing the amount of the analyte or compound which is measured and the denominator containing the amount of sample or some property of the sample in which the analyte is found. Most analytical effort is devoted to reliably measure the analyte portion of the concentration fraction and little effort is usually expanded on the sample part of the concentration fraction.

In humans or other animals, in order to estimate exposure or dose to chlorinated compounds including dioxin-like compounds, concentrations were historically measured in adipose tissue which contains most of the body burden of mammals. Values were expressed on a lipid extractable basis since no analytes are found in the polar water portions of tissues. However, adipose tissue collection is burdensome particularly in humans and use is made of blood analysis for estimation of exposure. Procurement of blood is easier than adipose tissue but the lipid content of blood is much lower (less than 1 %). It has been established for 2,3,7,8-TCDD[1] and the other 2,3,7,8-substituted PCDDs/PCDFs including the important TEQ value[2] and probably for the other dioxin-like classes that partitioning between adipose tissue and blood plasma/serum is close to 1:1 when both are expressed on a lipid basis. Thus in many instances in order to estimate body burdens of these contaminants, measurement of concentrations in blood lipid will suffice.

Lipids in biological samples can be arranged into two broad classes. One of these is the simple lipids consisting of esters of fatty acids with an alcohol. These include triglycerides (glycerol as alcohol), cholesterol esters (cholesterol as alcohol) and waxes (long chain aliphatic alcohols). This category is neutral in polarity and its function in organisms is mostly one of storage. The other class are the compound lipids consisting of esters of fatty acids with alcohol plus other functional groups. These

consist of phospholipids such as lecithin and glycolipids containing a carbohydrate with nitrogen e.g. cerebrosides. Compound lipids are more polar than simple lipids and their function is structural in nature. The lipids of adipose tissue are mainly the simple glycerides and those of blood plasma are more complex consisting of two simple lipids , fatty acid esters of cholesterol and triglycerides and the compound lipids, the phospholipids.

In our laboratories, the relative proportions of extracting solvent (ethanol, saturated aqueous ammonium sulfate, hexane) to plasma volume has varied depending on the quantity of the blood sample. For determination of background levels in the general population relatively large volumes of blood are used such as 100 mL with 100/100/300 mL of extracting solvent. For blood samples from exposed individuals smaller samples are usually available such as 2 to 10 mL and these are extracted with 25/25/50 mL of extracting solvent. The extraction of human plasma lipid from blood samples has been investigated with varying portions of extracting solvent. The effect on the lipid content and concentrations of dioxin-like compounds and PCBs are reported here.

Method

This study was carried out using two different pooled samples of human blood and the results reported on the average of the two pools. A relatively large aliquot (127 and 146 mL) of each pool was spiked with a mixture of ^{13}C-PCDDs/PCDFs/pPCBs and extracted with 100/100/300 mL of extracting solvent (ethanol/saturated aqueous ammonium sulfate/hexane) followed by 150 mL of hexane (method A). The total lipid content was measured gravimetrically for each pool. A second identical large aliquot of each pool as the first extraction above was spiked with carbon-13 internal standards and then subdivided into 10 equal parts. Each 12 to 15 mL aliquot was extracted with the same solvent mixture as before in amounts of 25/25/50 mL followed by 50 mL of hexane (method B) for a total of 10 extractions for each of the two pools. The extracted lipid from each of the 10 subfractions in method B was then combined and the total lipid of these two pools determined gravimetrically.
A small portion (less than 5%) of the lipid of both methods from each pool was used to determined the different classes of lipid using thin layer chromatography (TLC) on silica gel with flame ionization detection (FID) on the Iatroscan apparatus[3] . The remainder of the lipid of each pool from both methods was taken up in hexane, defatted, purified, and the dioxin-like compounds and PCB congeners determined as reported previously[4].

Results

Table 1 shows that total lipid content from both pools with method B (large proportion of extracting solvent to plasma) appears to extract only about two thirds of the lipid as method A (smaller proportion of extracting solvent to plasma volume). When the analytical values for PCDDs/PCDFs (reported as TEQ) and PCBs are expressed on a whole weight basis, the concentrations consisting of

the amount of analyte divided by the whole weight are the same for both methods. However when the concentrations are expressed on an extractable lipid basis, method A gives results lower by a third than method B. This suggests that the extracting solvent in both cases recovers all of the analytes being measured and that only a portion of lipid as determined gravimetrically is recovered with method B. The plasma lipid fractions from both methods A and B were analyzed by TLC-FID and these results are shown in Table 2. With method A, all three classes of plasma lipid (cholesterol, triglyceride, phospholipid) are detected and with method B no phospholipid is present.

Discussion

The more polar phospholipid lipid which normally consists of an important portion of the overall lipid in blood (but not adipose tissue) is not extracted into hexane when large amounts of ethanol are present. This lack of extraction gives a bias in the final concentration when values are reported on a lipid but not on a whole weight basis. In the situation where only small volumes of plasma or serum are available and relatively large volumes of extracting solvent are used, incomplete recovery of the polar lipid fraction in the blood samples has given an unreliable result when expressed on a lipid basis. As lipid contains the bulk of the human body burden, the latter parameter is overestimated to a considerable degree. This outcome underlines the necessity of defining not only the amount of analyte present in unknown samples but also characterization of the sample cannot be neglected.

References

1) Patterson D G, Needham L L, Pirkle J L, Roberts D W, Bagby J, Garrett W A, Andrews J S, Falk H, Bernet J T, Sampson E J, Houk V N (1988). Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 50 persons from Missouri. Arch Environ Contam Toxicol 17;139-143.
2) Schecter A, Ryan J J, Constable J D, Baughman R, Bangert J, Fürst P, Wilmers K, Oates R P (1990). Partitioning of 2,3,7,8-chlorinated dibenzo-p-dioxins and dibenzofurans between adipose tissue and plasma lipid of 20 Massachusetts Vietnam veterans. Chemosphere 20;951-958.
3) Oshsima T, Ratnayake W M N, Ackman R G (1987). Cod lipids, solvent systems and the effect of fatty acid chain length and unsaturation on lipid class analysis by Iatroscan TLC-FID. J Amer Oil Chem Soc64;219-223.
4) Ryan, J, Lau B P-Y, Boyle M J (1994b). Dioxin-like compounds in human blood. In Biological Mass Spectrometry: Present and Future, eds. Matsuo T, Caprioli R M, Gross M L, Seyama Y, John Wiley & Sons, Chp 3.16; 583-602.

Table 1: Effect of Solvent Volumes on Extraction of Dioxin-Like Compounds in Human Serum

Extraction media:

- ethanol, aqueous ammonium sulfate (AS), hexane

Methods:	Volumes			
Method	Serum	ETOH	AS	Hexane
A	100	100	100	300
B	10	25	25	50

Lipid Content

Method B/A	0.66	(n=10)

TEQ

	Whole Weight	Lipid Weight
Method A/B	0.96	0.66

PCB

	Whole Weight	Lipid Weight
Method A/B	0.95	0.60

Table 2: % of Lipid Classes in Human Plasma Extracted by Two Methods -

A: small volume solvent (~1:1)
B: large volume solvent (~3:1)

Class Lipid	A	B
CH-E	42	52
TG	16	26
FFA	--	--
CH	16	22
PL	26	--
Total	100	100

CH - cholesterol; E - ester; TG - triglyceride; FFA - free fatty acid; PL - phospholipid