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Utilization of Adipose Tissue Biopsy in Characterizing Human Halogenated Hydrocarbon Exposure

by Henry A. Anderson*

Halogenated hydrocarbons have been successfully utilized for pest control in agriculture and public health. In industry, the polychlorinated biphenyls (PCBs) have been particularly useful. Unfortunately, residues have proven persistent and have been found widely dispersed in the environment. Because they have chemical properties that favor bioaccumulation, it is not surprising that many have been identified in animals and man. Such findings prompted public health concern and initiated review of potential adverse health impacts. In many instances this process has led to total prohibition of use or strict limitations. Highly lipophilic, the primary accumulation site in humans is adipose tissue. Analysis of adipose samples remains the preferred biologic index for estimating exposure. Improved instrumentation has lowered the limit of detection and improved the accuracy of quantification. Accumulated population experience has helped develop understanding of the dynamics of tissue partitioning in humans. Once such relationships have been adequately described, other less invasive procedures may be more confidently used for general surveillance purposes. Whenever adipose tissue is obtained, the blood compartment should also be sampled and the relationship reported. We have only begun to investigate the resulting partition ratio as an investigative tool.

Introduction

For the past 50 years, many of the halogenated aromatic hydrocarbon compounds have enjoyed worldwide industrial and agricultural utility. These compounds have proven to be environmentally stable and highly persistent. Those which are least polar and resist metabolism and excretion readily bioaccumulate. Residues that have been identified in human biological specimens (Table 1) collected worldwide have raised public health concerns (*1,2*).

The most widely disseminated halogenated compounds which have received the most regulatory attention can be divided roughly into those used as pesticides and those with industrial applications: primarily the PCBs. Once the undesirable persistence and migration through soil, water, and into the human food chain was recognized, limiting additional environmental burdens of pesticides was more amenable to regulation and control than the circumstances surrounding PCBs. For pesticides the primary entry into the ecosystem was through the intended application of the product. In the United States and many other countries, these insecticides have either been completely removed from the market or severely limited in their use.

*Wisconsin Division of Health, P.O. Box 309, Madison, WI 53701.

Following the pesticide control model, restricting the manufacture and utilization of PCBs was the initial step toward limiting escape into the environment. However, unlike pesticides, vast quantities of PCBs remain in transformers and other manufactured products still in use and represent latent exposure potential. When these products require disposal, they will pose an additional contamination threat. Already disfunctional and discarded products exist in dumps and refuse sites which are now recognized as being unable to prevent escape of PCB containing leachate or other contaminated materials. Controlling future contamination of

Table 1. Halogenated aromatic hydrocarbons and their metabolites identifiable in human adipose tissue.

Aldrin	α-Benzene hexachloride
Dieldrin	β-Benzene hexachloride
Endrin	Lindane
Heptachlor	γ-Benzene hexachloride
Heptachlor epoxide	*o,p'*-DDT
Oxychlordane	*p,p'*-DDT
trans-Nonachlor	*o,p'*-DDE
Mirex	*p,p'*-DDE
Hexachlorobenzene	*o,p'*-DDD
Polychlorinated biphenyls	*p,p'*-DDD
Polybrominated biphenyls	Dioxins
	Dibenzofurans

the environment from existing, aging products and disposal sites has proven difficult.

The polybrominated biphenyls (PBBs) are not widely disseminated in the environment outside the State of Michigan. However, PBBs provide a useful study model; and considerable information is available from the epidemiologic evaluation of the contamination episode.

The dioxins and dibenzofurans fit neither of the previously mentioned categories. They have appeared in the environment as unwanted residual contaminants or waste by-products from the manufacturing of other less environmentally persistent chemicals. Accidents and fires in industrial and commercial buildings have also led to limited contamination. Preventing the entrance of these chemicals into the environment will be most difficult.

Evaluation of Techniques Utilized to Assess Exposure

Highly lipophilic, these compounds preferentially accumulate in adipose tissue. Depot fat functions as the primary storage site. Since the human body burden of these chemicals is in the adipose tissue, generally an adipose tissue sample has been the preferred biologic specimen for analysis. Serum and breast milk are the other frequently collected specimens which are utilized as proxies for body burden.

There are a number of distinct disadvantages to reliance upon an adipose biopsy for routine evaluation of exposure. Review of what we have learned over the past decade is a useful exercise as we plan future surveillance activities and refine the role for utilization of the adipose tissue biopsy (Table 2).

The foremost disadvantage is in the invasive and time-consuming procedure necessary to obtain the specimen. The least objectionable methodology is the percutaneous needle biopsy which, when the practitioner is experienced, can provide an average of 200 mg of wet weight lipid (*3*). The sample is usually obtained from the buttocks. In some individuals, especially the very thin and the elderly with poor skin turgor, this technique may be ineffective and produce insufficient tissue. Extensively utilized by the Environmental Sciences Laboratory of the Mount Sinai School of Medicine in the evaluation of the PBB episode in Michigan and studies of PCB workers, this method was found acceptable by most of the over 1200 subjects sampled. The procedure requires 20 to 30 min per sample. Observational and interview follow-up on a sample of the PBB exposed individuals found some morbidity, primarily bruising (greater than 5 cm), persistent pain, and discomfort at the biopsy site (longer than 48 hr) in 5 to 10% of the subjects. The PBB studies suggested that females had a greater propensity to develop extensive bruising.

Table 2. Utilization of adipose tissue biopsy.

Advantages	Disadvantages
Increased detectability of body burden	Invasive technique with risk
Can utilize autopsy/surgical material	Serial studies difficult
Descriptive epidemiology	Time-consuming for mass studies
Direct measurement of body depot	Clinical interpretation difficult

While serial study of the same individual is possible with this technique, subject motivation is crucial for success. Industrial or other high risk groups find the technique more acceptable than control or general population groups.

If a sample greater than 200 mg is needed because of technical laboratory needs (current dioxin analysis procedures usually request 10–20 g), the alternative is a surgical biopsy. This is a physician's office or hospital procedure most often requiring a follow-up visit for removal of stitches or clips from the biopsy site. Although morbidity has not been quantified, anecdotally it is reported to be similar to that of the needle biopsy with the added occasional permanent "dimple" at the site of tissue removal and the small incision scar. Resistance to serial study is expected to be greater than to the needle biopsy. Utilization of this method has been limited to small, selected high risk groups.

In the absence of overt clinical disease or the investigation of possible poisoning, interpreting the clinical significance of the analytical results is difficult. A result can be placed in descriptive perspective with other "general population" or occupational group values; but assigning a future disease risk is still quite speculative.

Despite these drawbacks, the adipose tissue biopsy remains the reference indicator of exposure tool and does have several distinct advantages over serum, whole blood, or breast milk which are the most commonly utilized biological materials gathered to assess exposure to the halogenated hydrocarbons.

Because of the favorable partitioning ratio (lipid to serum), the high lipid content of adipose tissue allows increased detectability of body burden. For example (Fig. 1), had serum PBB been used alone as an indicator of exposure in the PBB survey of the general population of Michigan, only 70% of the 839 individuals would have been considered exposed. When adipose tissue results are added, an additional 24% indicate exposure, raising the positive rate to 94%. Even though the limit of detection was an order of magnitude higher (2.0 ppb vs. 0.2 ppb in serum), the partition ratio of approximately 300:1 made the adipose limit of detection a more sensitive indicator of exposure (*4*).

This increased sensitivity is even more pronounced when the minimal contamination in the upper peninsula of Michigan was characterized. Only 19% of the serum samples were positive compared to 85% of the adipose samples.(*4*) However, when human exposure is high and nearly all serum samples expected positive, the added sensitivity of adipose samples is less important.

Another advantage of adipose over serum or breast

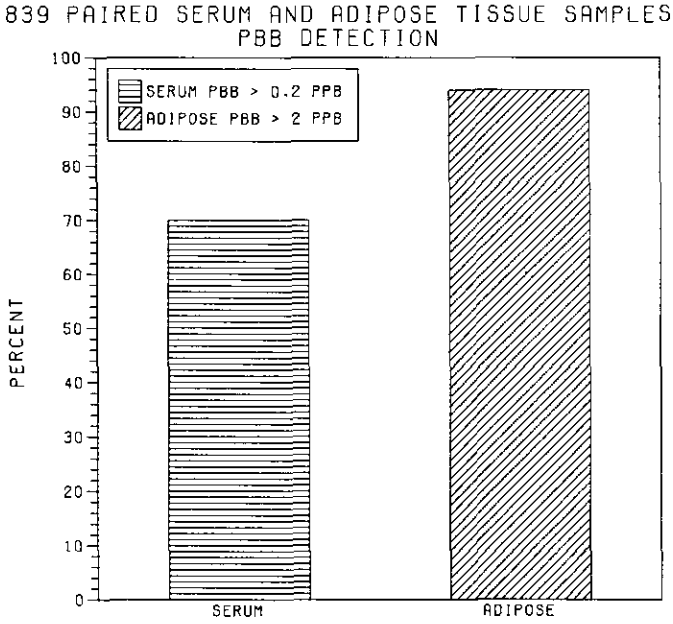

FIGURE 1. PBB detection: 839 paired serum and adipose tissue samples, Michigan general population.

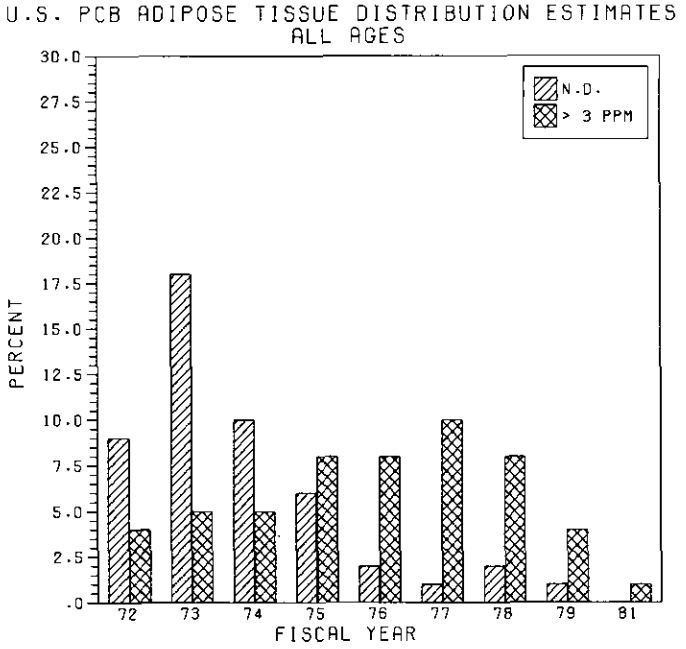

FIGURE 2. PCB adipose tissue distribution estimates; U.S.; all ages.

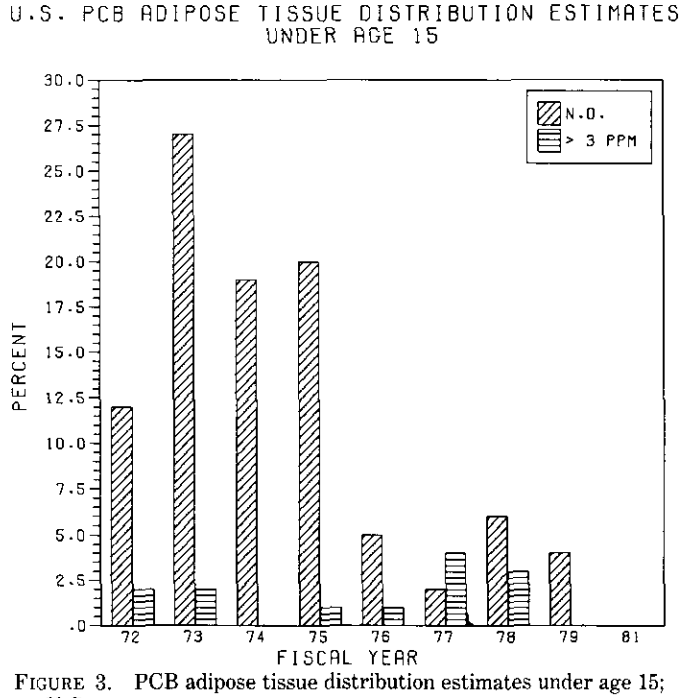

FIGURE 3. PCB adipose tissue distribution estimates under age 15; U.S.

milk is in the ability to utilize autopsy material or surgical specimens obtained incidental to other procedures. For surveillance programs, such passive systems are preferable. The National Adipose Tissue Survey of 20 halogenated compounds conducted by the Environmental Protection Agency's National Human Monitoring Program has effectively utilized this approach to monitor the prevalence and levels of chlorinated pesticides and PCBs in the general population of the United States (*5*).

Figure 2 summarizes the program's PCB data from 1972 through the first part of 1981. Visually, one can observe time trends showing a decrease in the percent of individuals with no detectable PCBs and a slight increase in the percent of those with PCB levels greater than 3 ppm. Despite the statistical limitations of the survey methodology, the program has begun to detect changes over time. Decreases for the compounds associated with DDT and increases for PCBs and similar compounds have been demonstrated through this national survey program.

More dramatic is the decline in nondetectables in the under 15 age group (Fig. 3). While the number of children with detectable levels has increased, the number with levels above 3 ppm is declining. However, it may be too early to view this trend from 1979 and 1981 data as definitive. Unfortunately, serial samples from the same individual are not possible, and thus any decline in the rate of individual accumulation of PCBs cannot be directly ascertained. Long-term surveys such as this play an important role in our assessment of population dynamics and efforts implemented to decrease exposure to these chemicals, and should be able to provide an indication that the rate of bioaccumulation has decreased or increased.

The National Adipose Tissue Survey is an excellent example of the type of descriptive epidemiology which has effectively utilized adipose tissue samples. Such observations have provided geographic, urban, and rural distributions and demonstrated that women have lower concentrations than men. Adipose tissue levels of halogenated hydrocarbons increase with advancing age.

Advancing from descriptive epidemiology to more analytic epidemiology investigating the relationships between adipose, serum, and breast milk concentra-

Table 3. Chlorinated aromatic hydrocarbon adipose tissue and serum partitioning ratios.

Compound	Exposure	Partition ratios	Reference
PBBs	Occupational: males	287:1	Wolff (*6*)
		190–260:1	Eyster (*7*)
	Farmers		
	Mixed sexes	300:1	Brilliant (*8*)
	Mixed sexes, ages	275:1	Wolff (*9*)
	Mixed sexes, ages	320:1	Wolff (*10*)
	Mixed sexes, ages	363:1	Landrigan (*11*)
	Pregnant females	140–180:1	Eyster (*7*)
	Nonpregnant females	193–230:1	Eyster (*7*)
	Males	325–329:1	Eyster (*7*)
	Michigan general population, mixed sexes	300:1	Wolff (*4*)
DDE/DDT	General population (DDE)	284:1	Morgan and Roan (*12*)
	Chemical workers (DDE)	230:1	Wolff (*6*)
	Farmers (DDE)	180:1	Wolff (*10*)
	Michigan general population (DDE)	160:1	Wolff (*4*)
Lindane	Occupational	195:1	Baumann (*13*)
PCBs	Occupational (Aroclor 1248)	120:1	Wolff (*14*)
	Occupational (Aroclor 1254)	160:1	Wolff (*14*)

tions has been hindered by the availability of comprehensive data sets. The apparent lack of dose response relationships in data sets which include health data and tissue concentration measurements has always been perplexing. Exposure ranges of four orders of magnitude have been described, but human health effects have not distributed consistently in the anticipated manner. The question has been raised whether the current methodology of reporting values accurately reflects toxicologic potential.

PCBs and PBBs are complex mixtures. Convention has determined which peaks relative to a commercial mixture will be quantified to arrive at the composite concentration used to describe the exposure. As discussed at this conference, over 40 PCB congeners have been identified in human adipose tissue, yet they are combined for descriptive purposes. Toxicologically they are far from identical. Current advances in analytic capability have allowed investigation to begin assessing individual isomers and how they distribute in human tissues.

The PBB contamination episode provided an opportunity to study large populations. Although some early investigations suggested serum and adipose tissue levels did not seem to correlate well with each other, the studies performed after continuing exposures had ceased and equilibration had apparently occurred did find excellent correlation between serum and adipose levels.

PCBs, DDT and DDE are more widely disseminated in the environment than PBBs. Table 3 summarizes most of the reports of partition ratios between adipose and serum. It is obvious that the PBB incident in Michigan triggered considerable research; the results of these activities have contributed greatly to our understanding of how halogenated hydrocarbons partition. With the exception of the group of pregnant and nonpregnant women, partitioning ratios for PBBs are quite consistent between studies and fall in the 200–300:1 adipose to serum range. The range for DDE is quite similar.

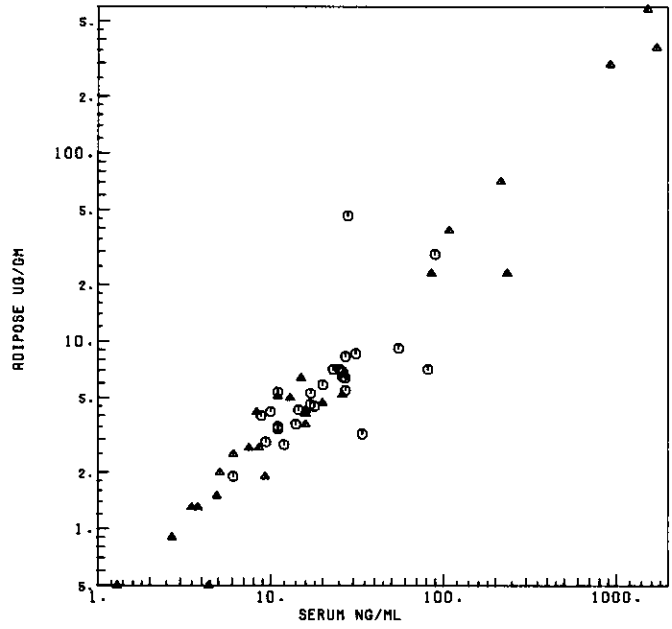

FIGURE 4. Paired adipose and serum samples: (Δ) PBB and (○) DDE concentrations.

Figure 4 displays the scatterplot of the 27 paired serum and adipose tissue samples from the 1976 survey of PBB exposed chemical workers (*6*). Both the PBB and DDE paired values are plotted. Although the range of PBB values is greater than for DDE, the two distributions visually appear indistinguishable. The intercepts for the two regression equations are 0.357 for PPB and 0.692 for DDE. However, at serum equal to 10 ppb, the ratio of adipose to serum is 304:1 for PBB and 349:1 for DDE. The regression lines are statistically indistinguishable.

Few paired samples have been reported for correlation of PCB partition ratios (Table 3). Wolff et. al.(*14*) reported three groups of partition ratios for PCB isomers from an occupationally exposed group. The ratios of adipose to serum ranged from about 80:1 to 200:1. The mixture of isomers in the exposure becomes important when interpreting the ratios observed.

Tuey and Matthews (*15*) have noted the similarity between the human adipose–serum PBB partition to that which is predicted by their pharmacokinetic animal model. Their model predicts PBB equilibrium at 340:1. The differences in the partition ratios reported by the different authors listed in Table 3 (*6–14*) may be a function of time since exposure. Experience with DDE and DDT suggest it may take several years for equilibration to have stabilized. Until equilibrium is reached, the ratios would be expected to be lower.

Unless acute, high exposures which may significantly alter equilibrium have occurred prior to testing, all studies reported a statistically significant correlation between adipose tissue concentration and serum. Sex, pregnancy and occupational status appear to affect the partitioning ratios.

Additional research will be needed to understand the significance of observing different partition ratios. Metabolism of congeners may differ as does toxicity. The ratio of adipose to serum may become an important factor in understanding disease potential. The laboratory precision in both the adipose and serum analysis adds variability. Sample size plays an important role in influencing the stability of the regression equation.

It may be that these factors combined could explain the different ratios observed. These types of relationships are being actively explored. Utilizing adipose samples in conjunction with other biologic specimens adds an important dimension to our understanding.

Conclusion

Unless identification of low exposures is required, the close correlation between serum levels and adipose tissue residues suggests that once the correlation is characterized, blood samples may be the best choice for surveillance and monitoring for these types of chemicals (Table 4). Whenever an adipose sample is obtained, unless from autopsy material, a serum specimen should also be included and paired results reported. The combination of these tissue analyses allows a better understanding of the dynamics and timing of an exposure. Partition ratios that are below those reported from groups expected to be in equilibrium may support a conclusion that an acute exposure is or has recently occurred. If this is confirmed, the partition ratio may be of utility in evaluating possible continuing environmental exposures, such as in the vicinity of toxic dump sites or other point sources of contamination.

Table 4. Effective utilization of the adipose tissue biopsy in the evaluation of halogenated aromatic hydrocarbons.

Cases suitable for adipose tissue biopsy
When detection of low level exposure is important
In conjunction with serum analysis, especially when evaluating suspected clinical poisoning
When autopsy or surgical tissue can be utilized for surveillance

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