A Selective Historical Review of Congener-Specific Human Tissue Measurements as Sensitive and Specific Biomarkers of Exposure to Dioxins and Related Compounds
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Estimating internal exposure or dose of dioxins and related chemicals such as dibenzofurans and dioxinlike polychlorinated biphenyls is relatively straightforward in laboratory animals because a known dose is given and the amount absorbed can be measured. In wildlife, direct tissue measurement and measurement of environmental samples have both recently been used to estimate exposure. Until recently, human studies used only indirect indicators such as skin lesions to qualitatively estimate exposure to these chlorinated organic compounds. Environmental measurements have also sometimes been used to estimate human exposure. Dioxins in human tissue were not measured until the 1970s, when 2,3,7,8-tetrachlorodibenzo-p-dioxin was measured in mothers’ milk; congener-specific measurement of dioxins and dibenzofurans in tissues (blood, milk, and adipose tissue) of the general population and exposed workers was first performed in the United States in the 1980s. Measurement in a sensitive and specific fashion of the 17 toxic dioxin and dibenzofuran congeners currently found in human tissue from industrial countries began in the 1980s. The use of known chemical standards, capillary columns, high resolution gas chromatography and mass spectrometry (GC–MS) has now become relatively common. GC–MS analysis of blood is currently accepted as the gold standard for estimating human exposure to dioxins. However, analyses are still costly and time consuming, and worldwide there are few qualified laboratories. There is currently a lack of knowledge concerning kinetics at higher and lower exposure levels for most of the toxic dioxin congeners and of levels in target tissues of concern. — Environ Health Perspect 106(Suppl 2):737–742 (1998).
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Historical Review

Estimation of dose of polychlorinated dibenz-p-dioxins (PCDDs) and related chemicals such as polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) is relatively straightforward in animal toxicology studies. Dioxins are usually administered by gavage or added to food. The percent of absorption is calculated from measurements of the labeled compounds. In oily vehicles absorption is quite high, approaching 100%, whereas absorption is usually less in aqueous vehicles (1–7).

For wildlife, congener-specific dioxin measurement in tissues, eggs, or environmental media is now frequently performed following discovery of a toxic end point such as eggshell thinning or malformations. Because synthetic chemicals other than dioxins are also sometimes elevated, the association between the individual chemicals in a mixture and the toxic end point may be less clear than in laboratory studies using specific dosing regimens in animal models (8,9). For example, in addition to dioxins, dibenzofurans, DDT, and DDT metabolites, many PCB congeners are elevated in tissue or egg specimens.

Until recently, human health studies used only indirect measures to demonstrate human exposure to dioxins. These indirect measures were neither sensitive nor specific. Erythema, a reddish discoloration of the skin, or chloracne, acne usually found in a group of workers following exposure to synthetic chlorinated organics, were the most commonly observed signs indicating exposure to chlorinated organics, including dioxins. Although Herxheimer (10) described chloracne in workers exposed to organochlorines in the 19th century, it was not established until 1957 that dioxins can cause chloracne (11).

The first human dioxin measurements were performed by Baughman in 1973 on breast milk collected in 1970 and 1973 (12). Baughman compared 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) levels in the milk of Vietnamese women from two villages sprayed with Agent Orange contaminated with TCDD to those of women living in Massachusetts. TCDD was not detected with the limits of detection available at that time in the milk of the women from Massachusetts. Figure 1 shows women from South Vietnam: these women had TCDD levels that, when lipid adjusted, were as high as 77 to 1850 ppt. The higher levels are from milk collected in the last year of known U.S. Air Force fixed-wing

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Abbreviations used: GC–MS, gas chromatography–mass spectrometry; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenz-p-dioxin; PCDF, polychlorinated dibenzofuran; PCP, pentachlorophenol; PeCDF, pentachlorodibenzo-razofuran; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Figure 1. Levels of TCDD in milk lipid from Southern and Northern Vietnamese women collected from 1970 to 1988 (13).
Agent Orange spraying (1970); the lower levels are from milk collected in 1973 (12–15). Figure 1 also shows TCDD levels in milk specimens collected in 1985 to 1988 from women living in Southern Vietnam and those collected in 1988 from women living in North Vietnam, where Agent Orange was not sprayed. The TCDD level is lower (2.1 ppt) for the milk of women from North Vietnam than it is for the milk of women from southern Vietnam (range 5.2–11 ppt). Archived Vietnamese milk specimens, which had been stored at −70°C at Harvard University (Cambridge, MA) by RW Baughman and M Meselson, were reanalyzed by J.F. Ryan in the 1980s using more refined modern techniques (16); the findings were almost identical to those previously reported by Baughman (12).

In the late 1970s, Rappe et al. (17) reported the presence of PCDFs in livers obtained from autopsies of two Japanese Yusho (rice oil poisoning) victims. In 1982 Rappe et al. (18) reported homologue groups of PCDDs and PCDFs in the blood of workers exposed to 2,3,4,6-tetrachlorophenate and in the blood of workers exposed to pentachlorophenol (PCP) or PCP derivatives (18).

Subsequent studies found that partitioning of dioxins varies between tissues even when reported on a lipid-adjusted basis (19). This complicates extrapolation from dioxin blood, milk, or adipose tissue levels to dioxin body burden. Figure 2, using U.S. general population data, illustrates that blood lipid generally contains the highest dioxin and dibenzofuran levels, followed by adipose tissue lipid, then milk lipid (20,27).

During the 1970s, environmental measurement of dioxins became more common and was sometimes used to estimate human exposure. In Seveso, Italy, Zones A, B, and R were designated as zones where high, medium, and slightly above background levels of TCDD, respectively, were found in soil near the site of a 1976 chemical factory explosion (22). Soil levels rather than human tissue levels were used to estimate exposure. Although initially useful, this approach did not take into account variability in bioavailability, differences in time of potential exposure, or differences in kinetics of absorption and elimination. Nor could it consider different intake scenarios such as dermal exposure and consumption of vegetables grown in home gardens potentially contaminated with TCDD. An exception to the exclusive measurement of TCDD in environmental samples was one autopsy performed with TCDD analysis of selected tissues from a woman who died of a presumably preexisting cancer shortly after exposure to TCDD in the Seveso incident. Adipose tissue had the highest TCDD level, followed by pancreas (embedded in adipose tissue), then liver (23).

In 1984 Gross et al. (24) published findings of elevated TCDD in recently obtained adipose tissue of Vietnam veterans highly exposed to Agent Orange contaminated with TCDD. The veterans’ exposure occurred between 1962 and 1971; the heaviest Agent Orange spraying occurred between 1967 and 1971 (24). Unlike some previous human tissue sampling for dioxin analyses, the adipose tissue was obtained by biopsy from living exposed veterans. Subsequent studies also found elevated TCDD in a relatively small percent of potentially exposed U.S. Vietnam veterans (25–30). Figure 3 presents the elevated levels of TCDD found in adipose tissue from six Massachusetts Vietnam veterans potentially exposed to Agent Orange (12,25). Also presented are estimates of TCDD levels at the time of exposure.

The heating of PCB and chlorinated benzene transformer fluid during an electrical fire on 5 February 1981 in Binghamton, New York, produced a mixture of PCBs, dibenzofurans, and dioxins (found in soot, surface wipes, and air) similar to that of the Japanese Yusho and Taiwanese Yu-cheng rice oil contaminations (31,32). Findings of elevated chlorinated dioxin and dibenzofuran congeners in the blood and adipose tissue was reported for the first time in 1983 in exposed American workers. Also noted for the first time was the presence of dioxin and dibenzofuran congeners in the

Figure 2. (A) Comparison of PCDD, PCDF, and PCDD/PCDF levels measured in blood, adipose tissue, and milk from the United States. (B) Comparison of PCDD, PCDF, and PCDD/PCDF calculated TCDD toxicity equivalent levels in blood, adipose tissue, and milk from the United States (20).

Figure 3. TCDD in adipose tissue (lipid 97%) of U.S. Vietnam veterans exposed to Agent Orange (13). *Calculated using three 6-year half-lives. †Adjusted for 1.4 increase in body fat.
fat and blood of U.S. adults from the general population, which served as the comparison group (31,33). The work was repeated and extended shortly thereafter with improved specificity and sensitivity of congener detection (34). Figure 4 shows total measured PCDD and PCDF levels from this study, which illustrates how the findings of elevated PCDF or PCDD congeners were used to document actual exposure in some potentially exposed Binghamton workers (12). Figure 5 shows PCB levels reported as Aroclor 1254 in the serum of other workers (firefighters) shortly after their exposure during the Binghamton incident and 10 months later (35). By 10 months after exposure, the PCB levels in the firefighters’ serum were similar to those of the general population. This illustrates the rapid decrease in PCBs frequently observed after acute exposure and demonstrates the potential usefulness of immediate as well as serial blood testing after exposure. Had PCB levels been measured only once, 10 months after exposure, there would have been no evidence of elevated levels, so erroneous conclusions regarding exposure could easily have been made.

Epidemiology studies in the 1980s, and even in the 1990s, rarely used tissue measurement to estimate exposure to or dose of dioxins. This omission caused difficulties in exposure assessment. Two of the former common assumptions were that the majority of Vietnam veterans had exposure to Agent Orange and its dioxin contaminant, and that the general population did not have any dioxin exposure. It was eventually realized that both exposure assumptions were incorrect (19,29,36,37). The New Jersey, Massachusetts, Michigan, and Centers for Disease Control Agent Orange exposure studies (25–30) showed that TCDD in blood or adipose tissue was elevated above U.S. background levels in only a small number of those Vietnam veterans studied who were thought to have been exposed to Agent Orange. It is now known that general populations, at least of persons currently living in industrial countries, have typical patterns of dioxins and dibenzofurans in blood and other tissues, although there can be variations in patterns by countries.

In the 1980s and early 1990s, exposure assessment studies also found that levels of dioxins are consistently higher in tissues from the general population of industrial countries and lower in persons from less industrialized countries (20,38). Elevated dioxin levels in heavily exposed workers have been demonstrated as long as 34 years after substantial exposure. Such extreme persistence of elevated dioxin levels demonstrates that human tissue dioxin measurement can be useful in documenting exposure for at least three decades after relatively high exposure (39,40). The association of PCDDs/PCDFs with relatively recent chemical industry synthesis of chlorinated organics was also demonstrated by the finding that levels of PCDDs/PCDFs in tissues of ancient (100–400-year-old) frozen Eskimo women from Alaska are very much lower than the levels currently found in humans from industrial countries (41).

Despite demonstration of the usefulness of tissue measurements and for a variety of reasons, many recent otherwise sophisticated epidemiology studies have not used tissue measurements of dioxins to estimate exposure (42–46). On the other hand, time employed in chemically contaminated work areas can sometimes be used successfully as a surrogate for exposure or dose in environmental epidemiology studies.

Some of the more recent epidemiology studies concerning consequences of exposure to dioxins began in the early 1990s to include human tissue measurements as an indicator of body burden (47–50). These studies were usually of cancer mortality and were stimulated by the seminal work of Hardell and colleagues (51–53). Hardell’s work pointed to a link between certain cancers and dioxin-contaminated herbicides and chlorophenols. The studies include reports of increased mortality rates for certain cancers and for ischemic cardiovascular disease (51–53). They also include reports of increased reproductive, developmental, and endocrine alterations in the children of mothers with higher general population levels of dioxin (49,50). The studies of the Japanese Yusho rice oil incident, which occurred in 1968, reported an increase in birth defects, certain cancers, and cancer mortality (54,55). In the almost identical Taiwanese Yu-cheng rice oil poisoning, which occurred in 1979, an increase in birth defects and cognitive and behavioral impairment was reported in children born to mothers with Yu-cheng poisoning (56).

Initially both studies estimated dose by measuring PCBs, PCDFs, and PCDDs in the rice oil and estimating the amount of rice oil consumed. Later reports on Yusho and Yu-cheng included human tissue measurement of PCBs and PCDDs/PCDFs (55).

Conclusions

Obtaining human tissues for dioxin measurements was difficult initially because adipose tissue or milk was necessary to provide a sufficient amount of the lipophilic dioxins to measure. This required either
minor surgery (usually under local anesthesia) in the case of the fat tissue, or a nursing mother in the case of milk. With improved analytic methods, blood has become the tissue of choice. At first one 450-ml unit of blood, usually 0.3 to 0.6% lipid, was required for blood dioxin measurement. At present as little as 50 to 100 ml of blood is needed for most general population (from industrial countries) dioxin analyses. However, obtaining 100 ml of whole blood requires a healthy adult or multiple blood samples from a child or less-than-healthy adult. Less blood is needed when dioxin levels are elevated above current background levels.

Dioxins have long half-lives of elimination in humans and for the most part they remain in human tissue in the parent form rather than as metabolites. Therefore, direct tissue dioxin measurement is useful for a considerable time after exposure if the level of exposure was sufficiently high or the exposure was prolonged. Knowledge of half-life of elimination for the 17 dioxin and dibenzofuran congeners commonly found in human tissue is critical in interpreting levels found from previous exposure. TCDD is currently believed to have a half-life of elimination in humans of between 6 and 11 years (57-61), whereas some higher chlorinated dibenzo-p-dioxins have been reported to have half-lives of elimination between 1 and 5 years (62-65). Górski et al. (66) reported half-lives of between 3 and 7 years for three PCDDs and half-lives of just over 1 year for two PCDFs. Recently, Flesh-Jansy et al. (58) reported half-lives for seven toxic PCDD congeners and six toxic PCDF congeners. They calculated that half-lives for dioxins range from 3.7 years for 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin to 15.7 years for 1,2,3,7,8-pentachlorodibenzo-p-dioxin. For dibenzofurans, Flesh-Jansy et al. (58) calculated that half-lives range from 3.0 years for 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin to a surprising 19.6 years for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF). Ryan et al. (62) reported a more rapid elimination of the Yusho-Yu-cheng higher chlorinated PCDFs (PeCDF and 1,2,3,4,7,8-hexachlorodibenzofuran) a short time after exposure when the levels were higher, and slower rates of elimination later as levels declined. They report half-lives of 2.0 to 2.5 years closer to the time of exposure when the PCDF levels were higher and of approximately 10 years when the levels were lower (62).

Relatively high cost and a relatively small number of available laboratories with demonstrated capability to accurately report down to the low parts per trillion background range have limited the use of congener-specific and -sensitive dioxin analyses in human health studies. Time for completion of analyses is relatively long compared to that required for other chemicals. The World Health Organization has certified fewer than 50 laboratories worldwide for analysis of PCDDs/PCDFs in human blood and milk. Some of these certified laboratories are government laboratories not available for nongovernment work. The charges of $2000 to $3000 per dioxin analysis have now generally decreased to just below $1000 each if done in quantity. Recently the dioxin analyses have begun to also include some of the dioxinlike PCBs.

Although gas chromatography–mass spectrometry (GC–MS) congener-specific dioxin analysis is generally considered the best available method of estimating exposure or dose, relatively fast and inexpensive biologic or other screening tests have long been sought. Many have been developed but to date none are in general use. These include tests designed to screen for aryl hydrocarbon receptor-type chemicals, currently estimated to cost $200 to $400 per test, with results obtained in less than 2 weeks. These screening tests include in vitro enzyme induction, use of monoclonal antibodies, and in vitro cell proliferation (67–74). The goal of such screening tests is to permit human tissue or environmental samples to be rapidly screened for evidence of elevated levels of dioxinlike compounds. Specimens with no evidence of elevation would be excluded from further analyses. Specimens with evidence of elevation would be analyzed by GC–MS for congener-specific information. Such screening could be useful after potential exposure of large numbers of people. It could, for example, facilitate screening following potential exposure of Vietnamese, U.S., and other Vietnam veterans (from Korea, Australia, and elsewhere) to Agent Orange. Three million Americans served in Vietnam during the Vietnam War, and millions of Vietnamese lived or still live in or near areas sprayed with Agent Orange. Exposures to dioxins, dibenzofurans, PCBs, and related chemicals can be of concern for workers, persons living near contaminated sites, and fish-eaters consuming dioxin-contaminated fish. At the present time and with only a few exceptions (75–78), most researchers consider GC–MS the gold standard for documenting increased dioxin dose.

It is hoped that all future health studies will use the refined exposure estimates now available. Such improved exposure assessment should markedly improve epidemiology studies relating to the health effects caused by dioxins.

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