The Caffeine Breath Test and Caffeine Urinary Metabolite Ratios in the Michigan Cohort Exposed to Polybrominated Biphenyls: A Preliminary Study

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A field biochemical epidemiology study was conducted using the Michigan cohort consisting of 51 rural residents exposed to polybrominated biphenyls (PBB). The study had three major objectives: a) to determine the serum half-life of the major PBB congener, hexabromobiphenyl (HBB), in the human, b) to determine if the PBB-exposed subjects had elevated cytochrome P-450I function as determined by the caffeine breath test (CBT) and the caffeine urinary metabolite ratio (CMR), and c) to determine the applicability of the CBT and CMR in field studies. PBB serum levels were detected in 36 of the 51 PBB-exposed subjects. The serum half-life of HBB was determined by comparing the current serum HBB values to the subject's previous serum values obtained 5 to 8 years earlier. The median HBB half-life was 12 years (range 4-97 years). The CBT and CMR were elevated in the subjects exposed to PBBs as compared to the values obtained from urban nonsmokers and were similar to those found in adults who smoke. A gender effect was seen in the PBB-exposed subjects, the median CBT and CMR values of the females being lower than the values of males. There was a correlation between the CBT and the HBB serum values ($r^2 = 0.2$, $p = 0.01$) but not between CMR and HBB serum values. The CBT and CMR were easily conducted in the field and appear to be useful metabolic probes of cytochrome P-450I activity in human environmental toxicology.

Introduction

Contamination of the Michigan food chain with polybrominated biphenyls (PBBs) occurred between July of 1973 and May of 1974 when approximately 295 kg of a mixture of PBB congeners, manufactured as a flame retardant (FireMaster FF-1), was accidentally added to dairy cattle feed (1,2). The contaminated feed was subsequently shipped to farms and retail outlets in Michigan, resulting in the dissemination of the PBBs in the Michigan food chain.

This PBB exposure had widespread effects. Contaminated cows had a dramatic reduction in feed consumption and milk production, along with other clinical signs, which eventually led to the identification of the chemical contamination (3). Many farms using the contaminated feed were quarantined, and 30,000 cattle, 2,000 swine, 400 sheep, and 2,000,000 chickens were destroyed and buried. A sample human population study (4) estimated that over 97% of the residents of Michigan's lower peninsula had detectable PBB concentrations in their adipose tissues. The humans with the highest PBB fat and serum levels were from families of quarantined farms who ate their farm products, individuals who obtained their food directly from these farms, or chemical workers who manufactured the PBBs (4,5). The Michigan Department of Public Health established a statewide 4,000-person PBB cohort made up of individuals primarily from the populations with the highest PBB exposure (5).

The impact of the PBB exposure on human health is a major concern due to PBB's long half-life in animals and presumed long half-life in humans and PBB's known biologic and toxic effects in animals including cancer (6,7), birth defects (8), hepatic damage (9), and altered immune (10) and reproductive (11) function. The exposed human population

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has been examined for potential PBB-induced toxicities by many different investigators, as recently reviewed (1,2). Although some studies have suggested impaired liver, immune, or neurological function, these effects have not been corroborated, and a conclusive correlation between the PBB exposure and biologic effect has yet to be demonstrated.

If a PBB-induced effect is to be found in the human, the liver may be the most sensitive organ to monitor. The liver, in animal studies, has been shown to be an important and sensitive sentinel organ to monitor PBB-induced biologic and toxic effects. The liver is the organ where PBBs are preferentially stored, is effected by PBBs at the lowest body burden, and is altered more by PBBs than any other organ showing dramatic histologic and functional changes (1,2,9,12–14).

Despite the liver being a sensitive sentinel organ of PBB exposure and toxicity in animals, hepatic function in PBB-exposed humans has been studied by only a few investigators using relatively insensitive biomarkers of liver function. In one study, liver serum enzymes were monitored in Michigan farmers exposed to PBBs and in Wisconsin farmers not exposed to PBBs. The Michigan population had a higher incidence of abnormal serum liver enzymes than the Wisconsin subjects (15). However, in a second study, serum liver enzyme values were within normal range in farmers and chemical workers exposed to PBBs, but enlarged livers were identified by physical examination in some subjects (16). These serum liver enzymes have been shown in animals to be insensitive indicators of polyhalogenated biphenyl-induced altered hepatic function (1,2).

The hepatic parameter in animals that appears to be the most sensitive biomarker of polyhalogenated biphenyl-induced biologic effect, including toxicity, is the cytochrome P-450-dependent mixed function monooxygenase (P-450) system (2,12–14). The mammalian P-450 system is a super gene family of at least eight enzyme families that are found in all tissues and is responsible for the metabolism of most environmental chemicals and the synthesis and metabolism of many endobiotics.

Of the eight P-450 families, family I and/or family II enzymes are induced by specific PBB congeners. Of these two P-450 families, the P-450I enzyme family in animals is the most sensitive and important biomarker to monitor PBB-induced effect (1,2). In fact, the P-450I enzyme(s) activity is altered at the lowest dose of PBB before any other hepatic or nonhepatic biomarker is affected (1,2,12–14). Equally important for its role as a potential biomarker, P-450I activity induction by many polyhalogenated biphenyls can be predictive of its potential to be toxic and some species' or individual's susceptibility to select chemical-induced toxic effects. For example, the most toxic congeners of polyhalogenated biphenyls are generally also the most potent inducers of the P-450I isozyme(s) activity (1,2,17). Conversely, the induction of P-450I function in certain strains of animals by select polyhalogenated biphenyls is an indicator of the animal's genetically controlled susceptibility to develop chemical-induced toxicities (1,2,17).

In the human, P-450I activity may also be a good sentinel biomarker of polyhalogenated biphenyl effect. Several investigators have shown that polychlorinated biphenyls and polychlorinated dibenzofurans can induce placental P-450I function and the degree of induction correlated with a decrease in birth weight (18). However, no study has examined hepatic in vivo P-450I activity of humans exposed to any polyhalogenated biphenyl including PBBs, even though the value of such human studies has been identified for many years (2,19).

In this pilot study, caffeine was used as a metabolic probe of P-450I activity. P-450I-dependent caffeine 3-N-demethylase activity was monitored by the caffeine breath test (CBT) (20), and 7-N-demethylase activity was monitored by the caffeine urinary metabolite ratio (CMR) (21).

The objectives of the study were as follows: a) to identify the estimated serum half-life of the major PBB congener, hexabromobiphenyl (HBB), in humans; b) to determine if the P-450I activity in Michigan rural residents exposed to PBBs is increased as compared to subjects not exposed to PBBs; and c) to assess the capacity of the CBT and CMR to serve as metabolic probes in field epidemiology studies.

Methods

Michigan Volunteer Population

Volunteers were selected from the 4000-subject Michigan PBB cohort (5). All subjects were healthy; taking no prescribed medications for the previous 2 months or over-the-counter medications during the 2 days prior to the clinic visits; nonsmokers for at least the previous 2 years; at least 20 years old; caucasians of primarily Northern European extraction; and rural residents.

Subjects were selected from the PBB cohort using the subject's initial enrollment serum PBB, polychlorinated biphenyl (PCB), and DDT levels determined 5 to 8 years prior to the present study. An effort was made to select subjects whose PCB and 1,1-bis(4-chlorophenol)-2,2,2-trichloroethane (DDT) serum levels were nondetectable or very low. In order to obtain subjects with a wide range of PBB body burdens, invitations to participate in the study were extended to 50 subjects with previously high PBB serum levels and 50 with nondetectable or low serum levels. There were to be essentially equal number of males and females. This volunteer population was to be compared to a previously studied control population of healthy caucasian adult smokers and nonsmokers not exposed to PBBs from Chicago and Toronto.

Clinical Protocols

Potential adult volunteers received an introductory letter that briefly outlined the purpose, procedures, and risks of the study. All selected subjects were subsequently visited by a field epidemiologist who discussed the study and obtained a verbal commitment to partake in the study. One week prior to the study day, a letter was sent to the subjects to remind them of the appointment day and protocol requirements. Volunteers were asked to refrain from ingesting products which contained methyl xanthines such as chocolate or caffeine for 24 hr prior to the clinic visit and

not to eat or drink anything except water after midnight prior to the clinic visits, which were scheduled between 7:30 and 10 A.M.

Volunteers whose medical history met the protocol criteria and whose physical examination and urinalysis on the morning of the study were normal were entered into the caffeine study after signing the consent form. A 30-cc specimen of venous blood was drawn from all Michigan subjects for determination of serum PBB, PCB, and DDT concentrations, and the caffeine breath test was performed.

After the 2-hr CBT, the subjects were offered orange juice and rolls before leaving the clinic. The adult volunteers who were to conduct the CMR were told they could resume their normal diet, but to refrain from ingesting products containing caffeine or chocolate until final urine samples were collected 10 hr later.

Caffeine Breath Test

The caffeine substrate used to monitor caffeine 3-*N*-demethylation in the CBT was [3-^{13}C-methyl] caffeine (99% ^{13}C) synthesized by Cambridge Isotopes (Cambridge, MA) or by A. N. Kotake (20). The labeled caffeine dose, 3 mg/kg (up to a maximal dose of 250 mg), was dissolved in 20 mL of sterile water and ingested by the volunteers, followed by ingestion of a 20-mL water wash of the container.

All subjects sat quietly for 15 min before and throughout the CBT. A breath sample was obtained by having the subject blow into a plastic bag just prior to and after the ingestion of the labeled caffeine at 30, 60, 90, and 120 min. Twenty milliliters of the expired air was removed from the bag by a syringe and injected into a nonsterile, plain, nonsilicone-coated Venoject tube (Terumo Medical Corporation, Elkton, MD) for transport and storage. The $^{13}CO_2/^{12}CO_2$ ratio was determined by differential gas-isotope ratio mass spectrometry (12,23).

The labeled CO_2 exhaled was calculated as percent labeled dose exhaled per hour (22). The excess $^{13}CO_2$-exhaled/mmole $^{12}CO_2$ for the dose was determined and multiplied by the calculated CO_2 production rate (22,23). The 2-hr accumulative exhalation of labeled CO_2 was the CBT parameter calculated, since it is the best monitor of caffeine clearance in the adult (20). The CBT data from the Michigan PBB cohort was compared to the CBT data from our control groups of healthy urban Illinois adult smokers or nonsmokers without a history of PBB exposure (20,24).

Caffeine Urinary Metabolite Ratio

All subjects voided prior to ingesting the labeled caffeine. Just before the subjects left the clinic, they voided again, and their urine was stored on dry ice. The volunteers were given a 1-L plastic bottle containing 5 g ascorbic acid in which to collect their urine for an additional 10 hr. Most of the subjects were instructed to store the urine bottles in the refrigerator until it was picked up by staff, usually the next morning. The bottles were then placed on dry ice for transport to the laboratory. The total volume of the urine was recorded, and an aliquot was stored at -20°C until analysis.

The urinary metabolites were determined by HPLC, and 7-*N*-demethylase activity was monitored by determining the molar ratio of 5-acetylamino-6-formylamino-3-methyluracil and 1-methylxanthine and 1-uric acid to 1,7-dimethyl uric acid (21). The CMR data from the PBB cohort were compared to CMR values from control groups of healthy, white Toronto adult smokers or nonsmokers who did not have a history of PBB exposure (21).

Serum PBB, PCB, and DDT Levels

Blood samples from each subject were immediately centrifuged and the serum was removed and stored at -20°C. The PBB, PCB, and DDT serum concentrations were determined by gas chromatography and electron capture detection (25) by the Michigan Department of Public Health's Analysis Laboratory (EPA quality assurance approved laboratory for PCB, PBB, and DDT serum values, < 9.5% variation on control sample determinations). Current and previous values of PBBs in the Michigan cohort were based on HBB levels, the dominant PBB congener in FireMaster FF-1. Other congeners of PBBs were not found in high enough serum concentrations to be detected. PCB was quantitated on the basis of Aroclor 1260 standards. The detection limits were 1 ppb for PBB and DDT and 3 ppb for PCBs.

The HBB half-life was calculated by determining the elimination rate of HBB between the previous and current HBB serum level and the time required to reduce the previous sample to one-half the original value. The median HBB serum half-life for the group was calculated by using only the data from subjects with current serum HBB values greater than 5 pbb (slightly above the minimal detection capacity of the methods) and whose previous value was greater than the current value.

Statistical Methods

Data for the Michigan volunteers with and without detectable serum levels and the nonexposed urban populations were compared by the Wilcoxon rank test using ranked data in the General Linear Modeling (GLM) procedures of SAS (SAS Institute, Cary, NC). Correlation between serum PBB levels and all other variables including the CMR and the CBT were determined with GLM.

Results

Volunteer Population

One hundred adults from the PBB cohort who met the clinical protocol criteria were contacted. Seventy-one adult subjects initially agreed to participate, of which 66 individuals came to one of the 13 clinics held in Michigan during the summer of 1985. Of the 66 subjects who attended the clinic, 15 did not meet criteria due to medications taken during the previous 2 months (12 subjects), abnormal physical findings discovered at the time of the clinic visit (one subject with a cardiac arrhythmia, and another with cholecystitis),

or a history of intolerance to coffee (1 subject). The subjects with abnormal physical findings were referred immediately to their private physicians.

All of the 51 participants resided in rural Michigan, and the primary occupations of the study group was farming or homemaking on farms. All but one of the farmers were primarily dairy farmers, and many of the farmers also had grown cash crops. Most of the individuals had lived on PBB-quarantined farms or directly obtained some of their meat and milk from these farms. The participants ranged between 20 and 79 years of age, and 25 were females and 26 were males.

Serum Residue Levels

The HBB serum values of the Michigan subjects exposed to PBBs ranged from nondetectable to 860 ppb, with a median serum concentration of 12 ppb. Fifteen volunteers did not have detectable PBB serum levels. No correlation between serum PBB and subject age, weight, or serum PCB and DDT levels were detected. There was no difference in serum PBB, PCB, or DDT levels between males and females.

Serum HBB half-life was determined for the 15 females and 12 males whose PBB serum values met the criteria as previously discussed (Fig. 1). The median serum half-life was 12 years, with a range between 4.6 and 94.7 years. The serum half-life for females and males was similar.

Three volunteers had current HBB serum values higher than their previous serum values. Of these three volunteers, only one had lost any weight since their previous PBB serum determination, and that was less than 3% of the total body weight.

Serum PCB levels ranged between undetectable and 99 ppb, with 4 individuals having a serum value greater than 10 ppb and 32 subjects having nondetectable PCB serum levels. The serum total DDT levels in the subjects ranged between nondetectable and 22.4 ppb. The serum DDT levels were greater than 10 ppb in 7 subjects and nondetectable in 5 subjects.

Caffeine Breath Test

The CBT values for the 51 PBB-exposed Michigan subjects (with and without detectable PBB serum levels) and our previously reported adult urban control groups of 28 healthy Illinois nonsmokers and 16 smokers (20,24), all of whom had not been exposed to PBBs, are shown in Figure 2. The PBB-exposed subjects had higher CBT values as compared to the urban nonsmokers (p = 0.02), but had CBT values not different from the urban smokers.

A comparison between the CBT values and the corresponding PBB serum values of the PBB-exposed subjects separated according to gender is shown in Figure 3. The subjects with and without detectable PBB serum levels had similar CBT values (median CBT value 5.0% and 5.3% for the 2-hr accumulative exhaled dose, respectively). There was a correlation between the serum PBB levels and the CBT values (r^2 = 0.2, p = 0.01). In the PBB-exposed subjects, the CBT values were greater in the males than the females (median CBT values 6.6% and 4.3% dose exhaled per 2 hr, respectively, p = 0.05).

Caffeine Urinary Metabolites

The CMRs were determined in only 44 of the PBB-exposed subjects because 7 subjects did not complete the urine collection or ingested caffeine prior to the end of the urine collection. The CMR values of the Michigan subjects, as compared to the CMR values from 27 unexposed adult smokers and 40 nonsmokers from Toronto [previously reported (21)], is shown in Figure 4. The CMR values in the PBB-exposed subjects were higher than those of the urban nonsmokers who were not exposed to PBBs ($p < 0.05$). In the PBB-exposed subjects, the CMR values were higher in the males than the females (median CMR values were 7.5 and 5.4, respectively, p = 0.01). There was no correlation between CMR values and PBB levels. The correlation between the CBT and CMR in the 44 subjects who completed both tests was significant (r^2 = 0.54, $p <$ 0.001).

Discussion

Thirty-six of the 51 PBB-exposed subjects had detectable PBB serum levels, and these serum levels were very high (4). The current median serum HBB level was 12 ppb, whereas less than 8% of the general Michigan population had serum levels above 3.1 ppb in 1978. Although 15 of the subjects did not have detectable levels, we assumed these subjects had PBB body burdens because 97% of all residents of lower Michigan in 1978 had detectable PBB concentrations in adipose tissue (4), and the subjects in the present study were from the Michigan cohort with the history of the largest PBB exposure. Fat biopsies would have been much more sensitive in assessing the PBB body burdens, as the adipose tissue contains approximately 300 times the concentration found in the serum (4), but the biopsies were not performed since most of the subjects would have refused to volunteer for such a study.
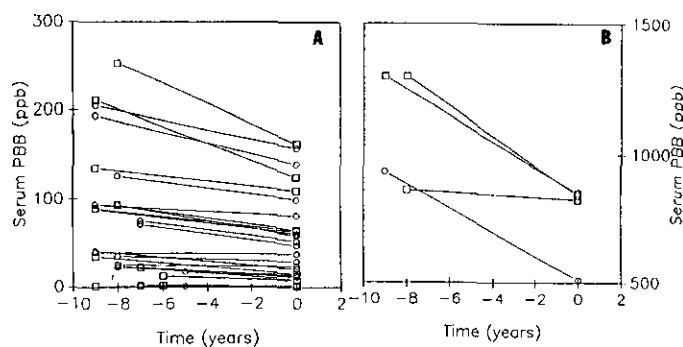

FIGURE 1. The alteration of the serum PBB (hexabromobiphenyl) levels with time. Time 0 represents the current PBB serum levels and the minus numbers on the abscissa represent the number of years prior to the current study that the previous serum samples were obtained. The data from the subjects with serum levels less than 300 ppb (A) and greater than 300 ppb (B).
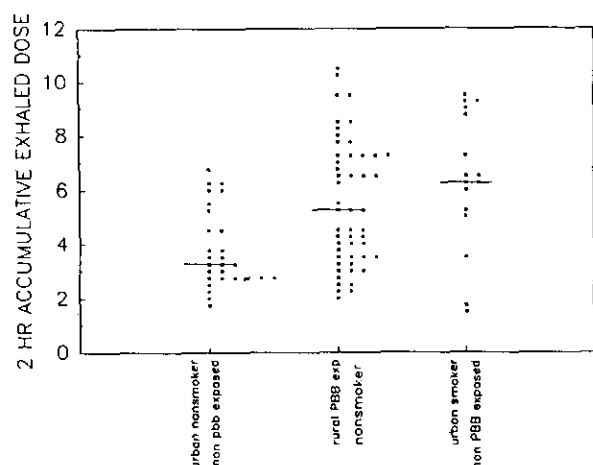
FIGURE 2. The distribution of the caffeine breath test values in the Michigan rural adults exposed to PBBs and the Illinois urban adult smokers and nonsmokers without PBB exposure. The horizontal line through each group's data points represents the median value of that group.
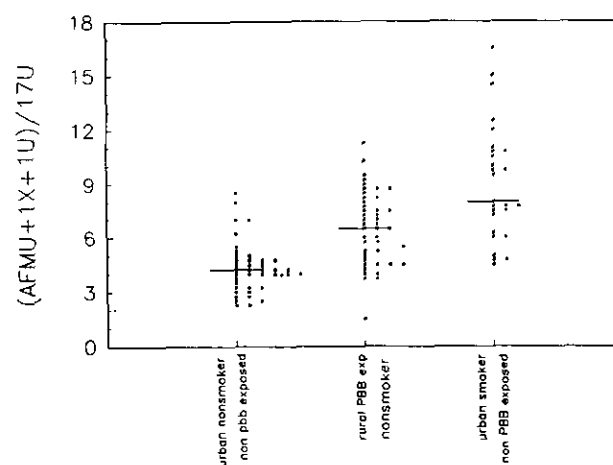

FIGURE 4. The distribution of the caffeine metabolic ratios values of the Michigan subjects exposed to PBBs and the Toronto smokers and nonsmokers not exposed to PBBs. The median value of each group is represented by the horizontal line.
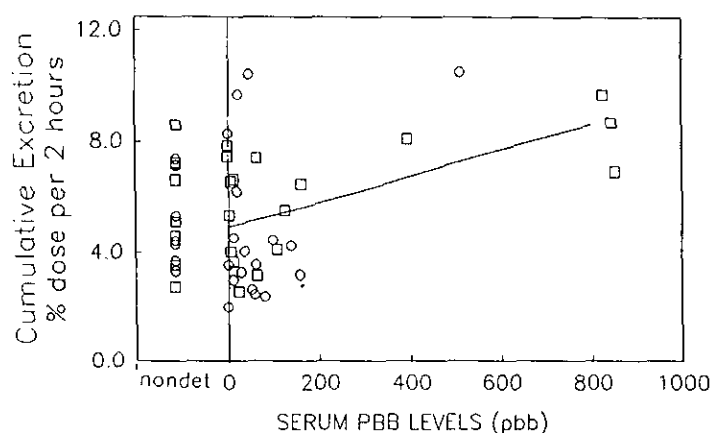

FIGURE 3. The correlation between the CBT and the PBB (hexabromobiphenyl) serum levels in the subjects exposed to PBB. (O) females; (□) males. The PBB-exposed subjects without detectable PBB serum levels are also represented on the left side of the graph (nondet). The regression line was calculated by using only the data from the subjects with detectable PBB serum levels (CBT = 4.96 + 0.0464 × PBB; r^2 = 0.2, p = 0.01).

The current and previous PBB serum determination were performed by the Michigan Department of Public Health Laboratory using essentially the same standard method (25). The gas chromatography assay monitors all PBB congeners, but the congener routinely monitored and reported was the HBB (25), since it is the major congener in FireMaster FF-1 and the only measurable PBB congener in most of the serum samples.

The calculated median HBB half-life was 12 years. The serum half-life can only be considered an estimate because serum PBB levels in an individual can fluctuate from week to week due to factors yet identified (Humphrey, unpublished data); the time between the two serum determinations was only 5 to 8 years, and half-life estimates are most accurate when based on observing the serum levels over at least

1.5 half-life (18 years in this case), and there may be continued PBB exposure of some of the subjects since three subjects (without significant weight loss) had slightly increased current serum PBB levels as compared to their previous serum levels. Despite all the potential confounders, the observed 12-year median half-life calculated in this study is the only data available from humans studied over so many years. The previous estimate for PBB half-life was 6.7 years (26), and this was based on data derived from rodent studies.

The subjects used for comparison to the rural Michigan PBB-exposed subjects were urban Chicago and Toronto caucasian smokers and nonsmokers whose data have been previously reported (20,21,24). The PBB, PCB, or DDT serum levels of the urban residents were not determined at the time they were studied. However, the subjects should not have had any PBB or abnormally high PCB body burdens since the PBB contamination did not extend to Ontario or Illinois; nor had the subjects eaten unusual quantities of Lake Michigan fish, the primary source of high PCB exposure in the Great Lakes area. All subjects of each group were caucasian but were not matched for age or gender; however, we and others have not found gender- or age-related changes in the CMR or the CBT (20,21,28). The groups were not studied simultaneously, but the serum determinations and methodology were well controlled and standardized (as discussed earlier), and the CBT has been shown not to vary over 10% in the same subjects repeatedly studied during an 18-month period (28).

A central objective of the study was to monitor the P-450I activity in PBB-exposed subjects and to determine if PBBs induce the isozyme(s) in the human. The CBT and CMR were developed to indirectly determine P-450I isozyme(s) activity by monitoring caffeine N-demethylation. A number of previous studies have shown the CBT and CMR monitors P-450I activities. In the rodent, caffeine clearance and the CBT are induced by polyaromatic hydrocarbons such as 3-methylcholanthrene that induce the P-450I isozyme(s), and they correlate very closely to hepatic P-450I-dependent

activity (r = 0.9) (29,30). Caffeine clearance, the CBT, and the CMR are induced in the human by smoking, and this induction has been used as evidence that the three methods are primarily monitoring polyaromatic hydrocarbon-inducible P-450I isozyme(s) (20,27,31). More recently, Campbell et al. (32) used human hepatic microsomes in a reconstituted microsomal system and demonstrated that caffeine N-demethylation is blocked competitively by benzo(a)pyrene and ethoxyresorufin, substrates that are metabolized by P-450I isozyme(s). There was also an excellent correlation between caffeine N-demethylation activity and P-450I-dependent ethoxyresorufin-O-deethylase activity in human hepatic microsomes.

The two methods employed to monitor caffeine N-demethylation were the CBT and CMR. The CBT monitors caffeine 3-N-demethylase activity, the primary route of caffeine metabolism (20), by determining the rate of exhalation of labeled carbon dioxide derived from the nonradioactive labeled [3-^{13}C methyl] caffeine. The CMR monitors 7-N-demethylation by comparing the molar ratio of urinary caffeine metabolites that were demethylated at the 7-methyl position to the metabolites that were not metabolized through the 7-N-demethylation pathway (21). The identification of which P-450I family isozyme is primarily responsible for 3- and 7-N-demethylation of caffeine will have to await the results of in vitro caffeine metabolic studies using pure human P-450I enzymes.

The PBB-exposed group's CBT and CMR values were increased in comparison to the values of the urban subjects not exposed to PBBs and were similar to the increased CBT and CMR values of the adult smoker. This induction occurred in exposed subjects with and without detectable HBB levels. This suggests that if the PBBs were responsible for some of the induction, the induction occurred at PBB body burdens below detectable serum levels and/or there were other chemicals in the rural environment that induced the P-450I enzymes. It is of note that previous studies examining PCB and polychlorinated dibenzofuran levels and placental P-450I activity reported the exposed groups had induced P-450I levels but there was no correlation between the chemical levels and P-450I activity (18).

The PBB exposure appears to at least partially induce P-450I activity in the human as it does in the animal, since there was a significant correlation between the increase in PBB serum levels and the CBT. We did not identify a correlation between PBB level and CMR, and this may be due to several reasons, including the fact that the subjects with the highest CBT values did not complete the CMR.

There are many reasons why a close correlation between PBB serum levels and P-450I activity may not be present. Genetically controlled factors of isozyme induction in the human may prohibit the induction of all subjects exposed to PBBs. These noninducible subjects appear to make up a considerable proportion of the population (20,27), thereby not permitting a close correlation. For example, if only one-half of the subjects were genetically capable of being induced, the maximal, r^2 value would be very similar to the 0.2 value reported in this study.

Another factor that may affect the correlation between

PBB serum levels and P-450I activity is that the major PBB congener, HBB, was measured in the serum and used as the indicator for the PBB body burden. This congener induces P-450II isozymes but not P-450I isozymes in rodents (1,2). If fat biopsies could have been performed, the fat level of other congeners, particularly the congeners that are potent inducers of the P-450I isozymes in animals, could have been monitored, and the correlation between these congeners and the caffeine tests may have been much closer.

The P-450I activities in the PBB-exposed subjects were lower in the female than the male subjects despite similar PBB serum values. The cause(s) for this gender-related difference is unknown, but oral contraceptives that are known (33) inhibitors of P-450 activity were excluded as a cause of this gender-related effect, since only two of the females reported they had taken oral contraceptives during the previous 2 months, and both had CBT values that were at the median value. The males could have been exposed to other chemicals capable of inducing the P-450I isozymes or the females exposed to chemicals in the home that could inhibit P-450I induction.

The apparent PBB-induced increase of the P-450I isozyme(s) activity in the subjects was less than 55%. This degree of induction was not as great as that seen in smokers or in animals exposed to PBBs (2,19). This relatively small induction may imply either the PBBs in FireMaster FF-1 were not potent inducers of P-450I isozyme(s), and/or that the human species is not very inducible by the PBBs. If the induction of P-450I isozyme(s) is an indication of potential toxicity, as it is in some animal species, than the PBB exposure in the human may result in little toxicity. The definitive data regarding the ultimate biologic effect of the PBB can only be answered decades or possibly generations from today.

The CBT and CMR may be valuable tools in biochemical epidemiology since both are safe, noninvasive, and can be readily performed in the field in subjects of all ages (24). A good correlation (r^2 = 0.54, p = 0.001) was observed between the CBT and CMR, and each test has its unique advantage and disadvantages (28).

In conclusion, the objectives of the study have been accomplished. The HBB serum half-life was estimated to be approximately 12 years, the PBB-exposed Michigan rural residents were found to have induced P-450I activity which was at least partially due to the PBB exposure, and the CBT and CMR were found to be easily conducted in the field as monitors of the P-450I biomarker. Other studies will be necessary to determine the polyhalogenated congeners that are P-450I family inducers and to further explore the significance, capacity, sensitivity, and specificity of the P-450I biomarkers in human environmental toxicology studies.

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