*Journal of Chromatography*, 239 (1982) 217–226
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,503

# LONG-TERM VARIATION STUDY OF BLOOD PLASMA LEVELS OF CHLOROFORM AND RELATED PURGEABLE COMPOUNDS

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## SUMMARY

Values for circulating plasma chloroform of 25 white adult females were monitored for 6 months during the first phase of a four-phase long-term variation study. The data suggested four major exposure categories. Category I (20%) had average chloroform levels < 10 ppb* and variation ranges ⩽ 10 ppb. Category II (24%) had average levels of 10–25 ppb and ranges of ⩽ 10 ppb. Category III (20%) had average levels of 10–25 ppb and ranges > 20 ppb. Category IV (28%) had average chloroform levels of > 25 ppb and variation ranges > 20 ppb. Although the participants had been carefully screened to exclude incidents of occupational and recreational exposure, three subjects in category IV experienced obvious incidences of acute exposure to either chloroform or a chloroform precursor. In these situations circulating plasma chloroform levels were between 1655 ppb and 4000 ppb.

## INTRODUCTION

Countless articles have been published concerning the presence of trihalomethanes in public drinking water supplies (for representative articles, see ref. 1), but very few reports have been made of the levels of these compounds in human tissues. This is not surprising in that the bulk of volatile halogenated organic compounds are readily expired, and most of the brominated species are effective alkylating agents which form non-volatile complexes with several blood components. Nevertheless, in 1979 Peoples *et al.*[2] published an analytical method by which volatile halogenated hydrocarbons can be purged from both human adipose tissue and blood plasma. The method, based on the purge/trap/desorb procedure of Bellar and Lichtenberg[3], has not been applied to many adipose tissue samples but has been used to analyze hundreds of blood samples in an effort to determine if a correlation exists between blood chloroform levels and the amount of chloroform found in the drinking water of individuals. Some correlation has been demonstrated[4] in that on the average, individuals who use finished water sources high in chloroform content do tend to have

* Throughout this article, the American billion ($10^9$) is meant.

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significantly higher plasma chloroform levels. However, to date, no report has been made of how plasma chloroform levels vary with time, and if the levels remain rather constant or scatter considerably over time for the same individual.

This report summarizes Phase I of a four-phase project (described below) concerned with determining long-term variations of human blood-plasma chloroform levels. Twenty-five white females were monitored over 6 months. Their average plasma chloroform levels and concentration ranges have been determined, categorized and reported herein.

## EXPERIMENTAL

The apparatus and experimental conditions employed in this investigation have been previously reported[2].

## RESULTS AND DISCUSSION

### *Analytical findings*

Between five and seven values were obtained for each of the participants, and 92% of the subjects fell into one of four categories, as indicated in Table I. Values for four individuals from each category have been included in Figs. 1–4. Fig. 5 includes three special subjects who form subset IV-E within category IV.

TABLE I

CATEGORIZATION OF PLASMA CHLOROFORM LEVELS

| *Category* | *Average $CHCl_3$ (ppb)** | *$CHCl_3$ range (ppb)* | *Specific examples* | *Percentage subjects*** |
|---|---|---|---|---|
| I | <10 | ≤10 | D, E, G, J, L | 20 |
| II | 10–25 | ≤10 | B, F, I, R, T, Y | 24 |
| III | 10–25 | ≥20 | H, M, O, U, W | 20 |
| IV | >25 | ≥20 | A, K, N, P, S, V, X | 28 |

* ppb = μg/l. or parts of chloroform per billion parts of plasma.

** One subject had an average $CHCl_3$ level of 10–25 ppb and a $CHCl_3$ range of 10–20 ppb; another had a level of >25 ppb and a range of ≤10 ppb.

Category I represents, from a health perspective, the best possible situation: consistently low exposure to chloroform as evidenced by low levels of circulating $CHCl_3$ or $CHCl_3$-precursor (discussed below) in the bloodstream; 20% of the participants fell into this category. Values for subjects D, E, J and L appear in Fig. 1. Subject L was remarkably consistent in that over a 5-month period she exhibited a total range of only 4 ppb, fluctuating only 1 ppb below and 3 ppb above an average chloroform level of 5 ppb. Subject G (not shown) was the most variable in this group and yet only fluctuated 3 ppb below and 5 ppb above an average $CHCl_3$ level of 9 ppb.

Category II represents the more usual levels of circulating chloroform in the bloodstream. It does not contain examples of acute exposure inasmuch as the fluctuation range is 10 ppb or less; 24% of the participants fell in this category. Values for

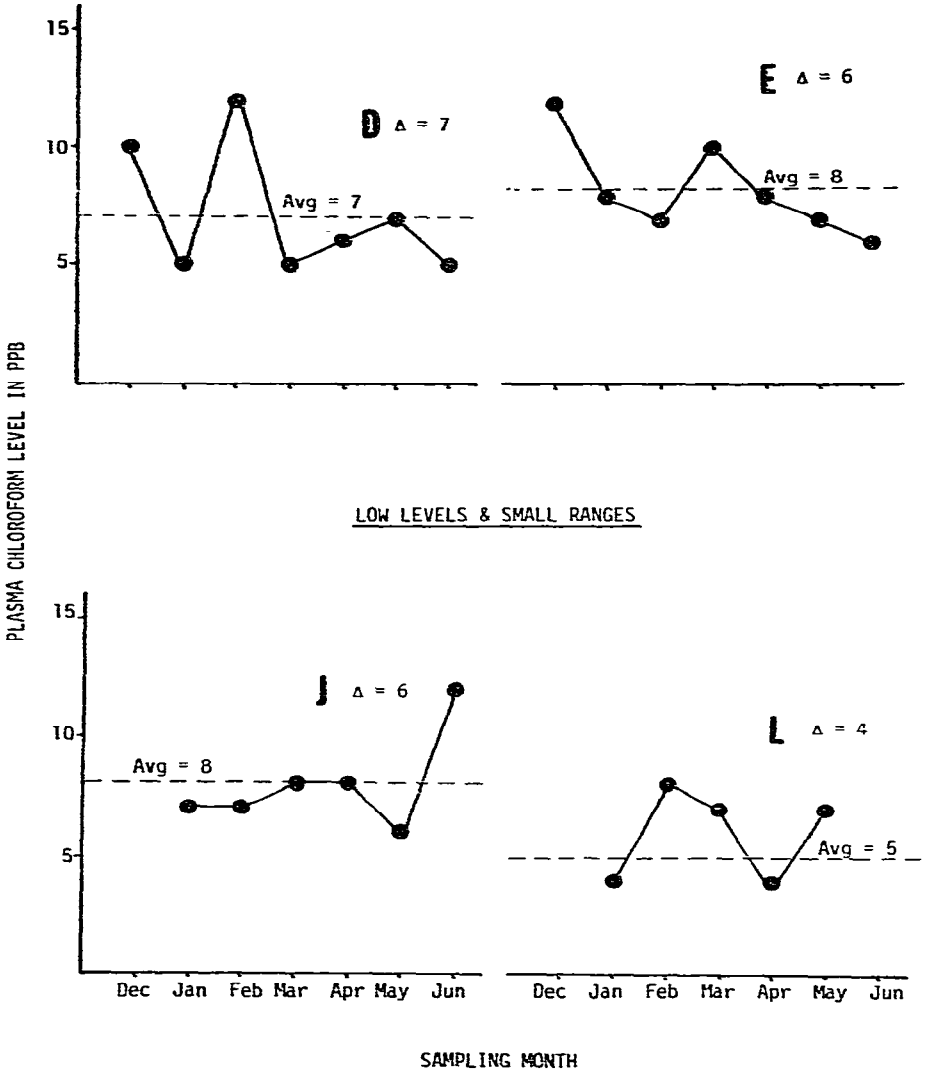



Fig. 1. Examples of values for category I participants. Plasma $CHCl_3$ levels are in parts per billion; ranges of $CHCl_3$ values are designated by $\Delta$. Averages are represented by broken lines; subject identities, by capital letters which cross-reference with the text.

subjects B, R, T and Y appear in Fig. 2. Subject I (not shown) was remarkably consistent in that over a 6-month period she had a total range of only 4 ppb, fluctuating above and below an average value of 10 ppb $CHCl_3$ by only 2 ppb. Subjects F and Y also had consistently constant levels; their fluctuations ranged over only 5 ppb.

Category III contained 20% of the participants, and represents exposure levels of 10–25 ppb of circulating chloroform and large fluctuation ranges. Values for subjects H, O, U and W appear in Fig. 3. Within categories I and II, ratios of the maximum level of $CHCl_3$ to the minimum level (max/min) were generally 2 or less. In

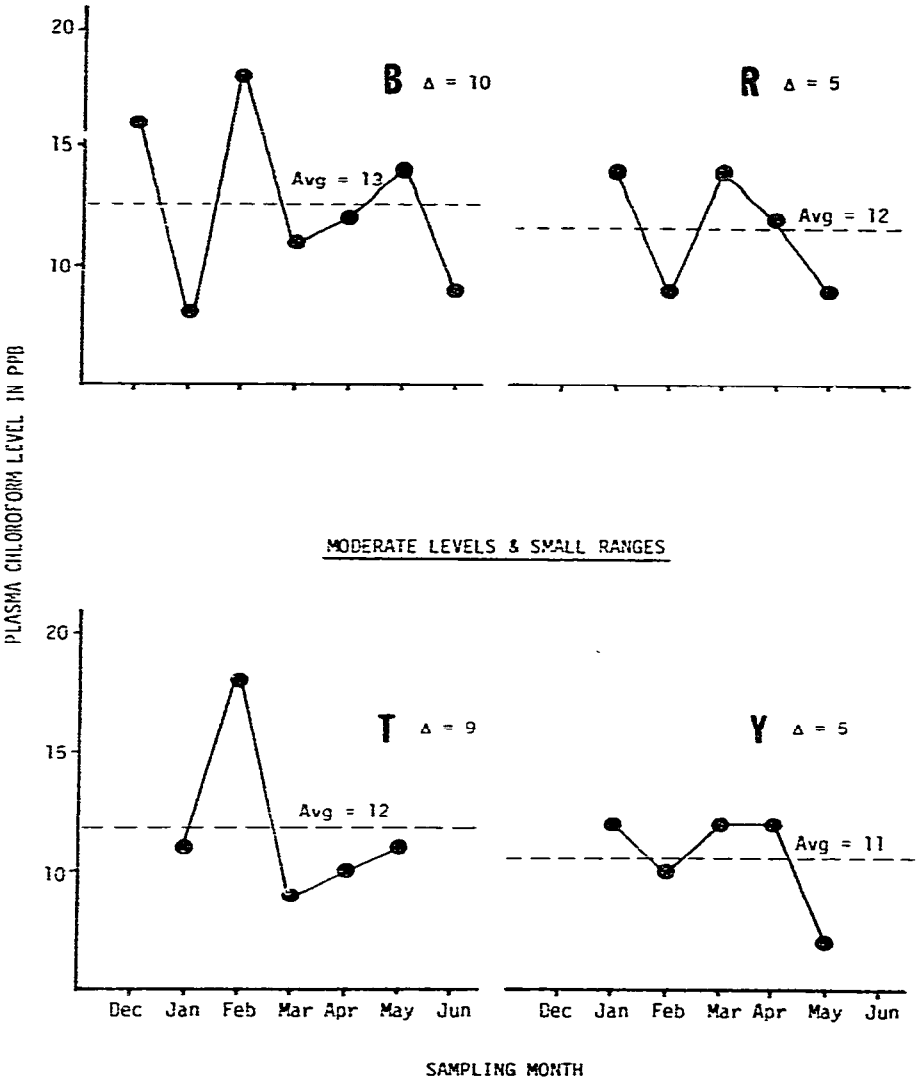
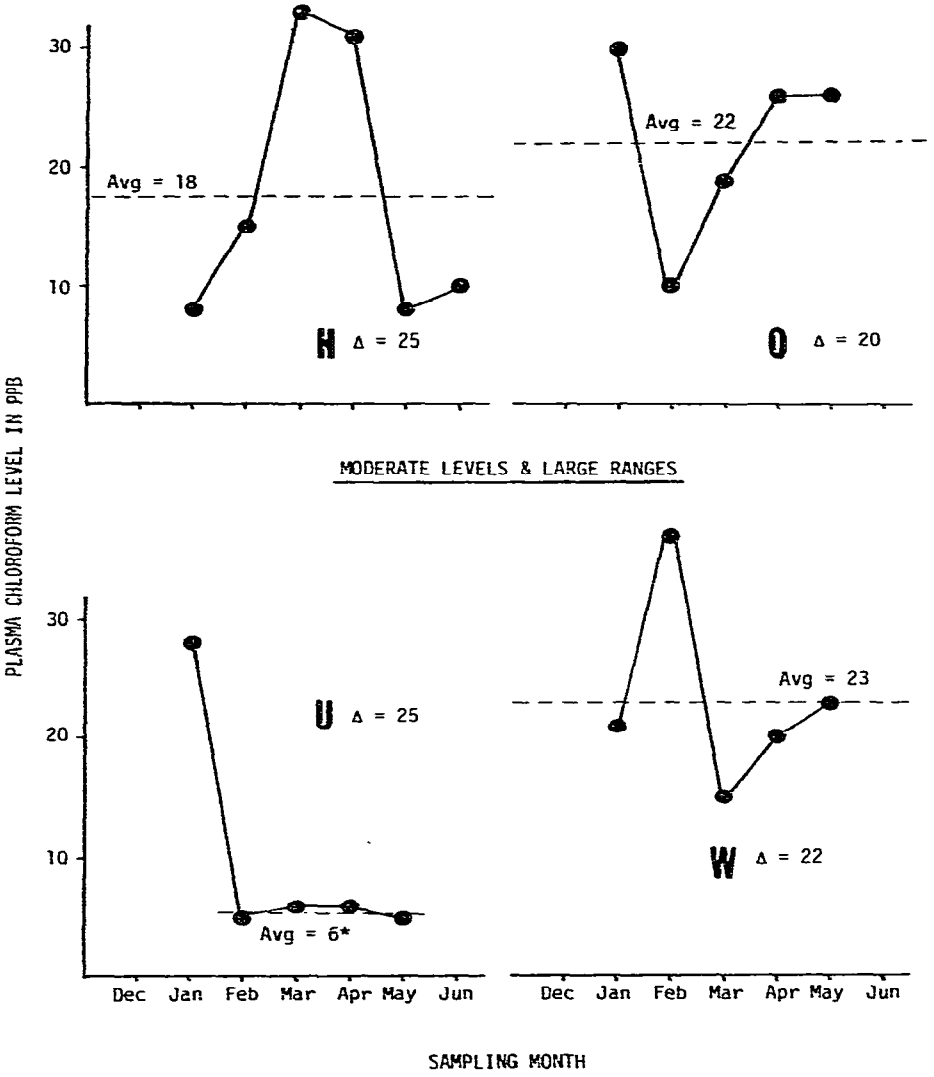



Fig. 2. Values for some category II participants. See Fig. 1 for further explanation.

category III, this ratio often exceeded 3, probably indicating some inconsistent exposure although not of a highly acute nature. Subject H appears to have passed through a sustained period of increasing chronic exposure. Subject O represents an almost consistent, cyclic fluctuation in this category.

Subject U was remarkably constant over a 4-month interval, varying only between 5 and 6 ppb, a 1-ppb range. The somewhat high level for subject U during January may be the down-side of a truly acute exposure situation. Subject W also exhibited an almost cyclic fluctuation of values which did not quite reach the three-fold difference for (max/min) obtained for the other members of this category.




*First point omitted in average

Fig. 3. Values for some category III participants. See Fig. 1 for further explanation.

Category IV contains some examples of sustained high levels of circulating chloroform (above 25 ppb) and high fluctuation of these levels (above 20 ppb). Consequently it includes examples of both sub-acute exposure (Fig. 4; subjects K, N, S and X) and acute exposure (category IV-E; Fig. 5; subjects A, P and V); 25% of the participants were in this category.*

Participants K, N (borderline), S and X probably do not represent acute ex-

* To graph the values in category IV, the *y*-co-ordinate axis was, in most cases, changed from the usual range of 0–30 ppb $CHCl_3$ to as large as 0–4000 ppb.

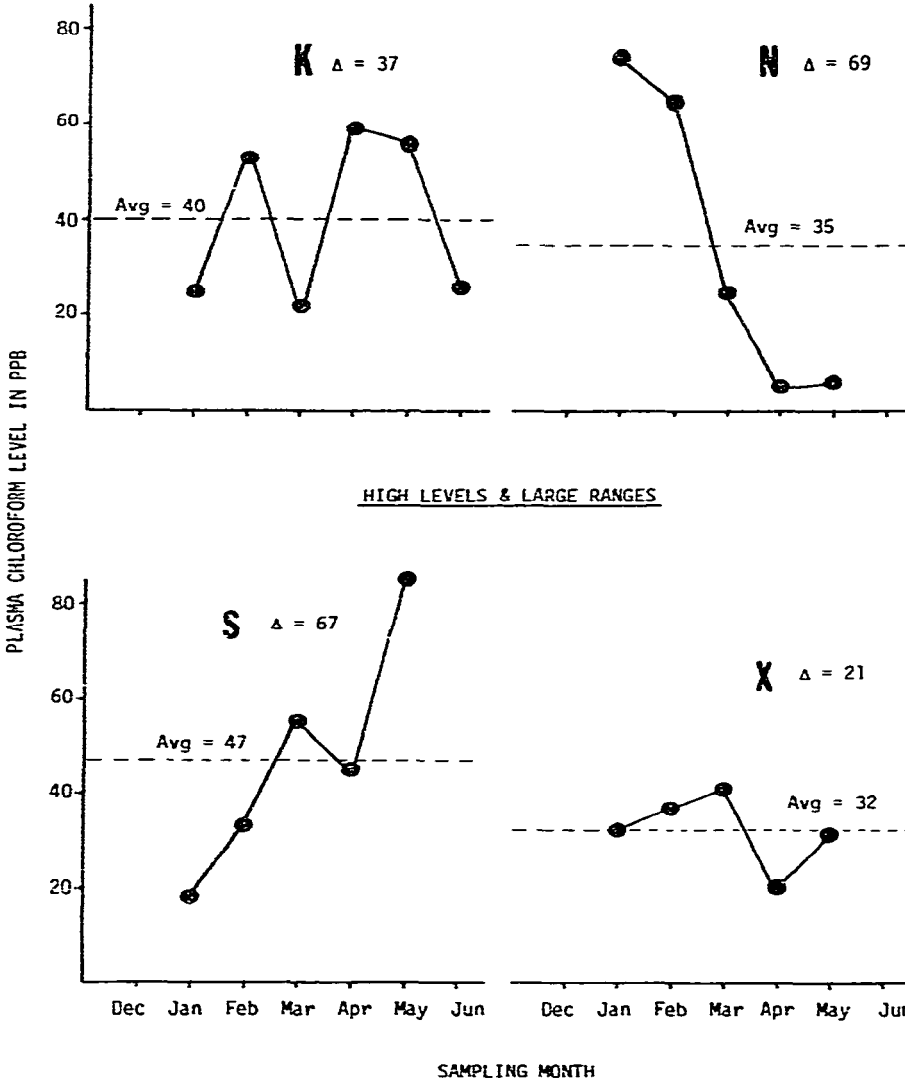



Fig. 4. Values for some category IV participants (non-acutely exposed). See Fig. 1 for further explanation.

posure situations inasmuch as their (max/min) ratios range from 2 to 5. They are, however, good examples of how widely $CHCl_3$ levels can fluctuate.

Subject K, which has an average chloroform value of 40 ppb, fluctuated between 22 and 59 ppb and actually passed through two cycles during the 6-month sampling period. Subject X was similar, with an average $CHCl_3$ value of 32 ppb and a range of 20–41 ppb. Only one cycle is evident for this individual.

Subject S, which averaged 47 ppb of $CHCl_3$, indicated a rather steady increase in $CHCl_3$ level from 18–85 ppb. Participant N showed a steady decrease from 74 to 5–6 ppb. The (max/min) value was 15.

Undoubtedly subjects A, P and V represent acute exposure situations (Fig. 5).

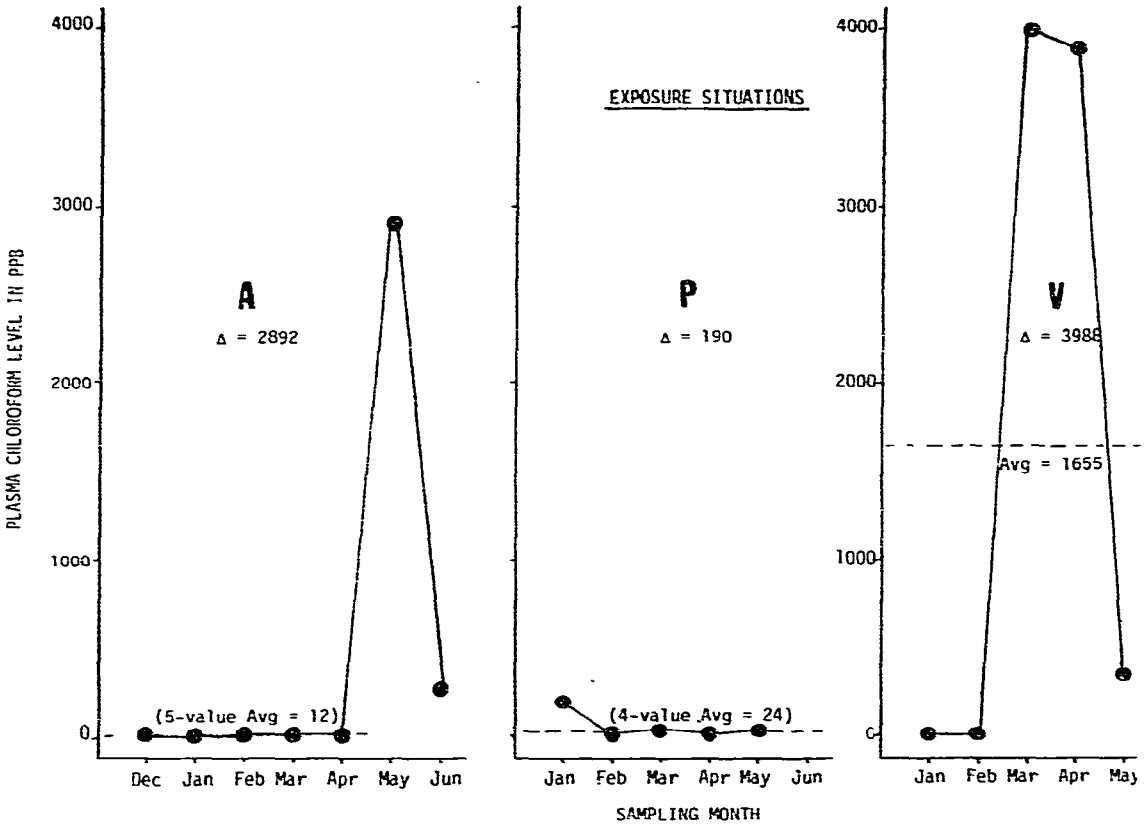



Fig. 5. Values for a subset of category IV participants (IV-E) who where acutely exposed to either $CHCl_3$ or a precursor thereof. Note the extreme change in the values along the $y$-coordinate axis. See Fig. 1 for further explanation.

For 5 months participant A exhibited a chloroform level of 8–18 ppb with an average value of 12 ppb. During May and June, however, the respective values were 2900 and 275 ppb. This subject had the highest (max/min) value observed, 363. Participant V was almost as high with a ratio of 333. After having $CHCl_3$ levels of 12 and 14 ppb for two successive months, levels of 4000, 3900 and 350 were obtained for the period March–May.

Subject P exhibited a rather constant level of chloroform, between 15 and 30 ppb (average 24 ppb) after an initial elevated value of 205 ppb. The (max/min) value was 14.

The extreme values found during Phase I work included: (1) chloroform levels from 4 to 4000 ppb (or 350 ppb if three values above 350 are excluded); (2) average chloroform levels from 5 to 1655 ppb (or 47 ppb if three values above 47 are excluded); and (3) total spread ($\Delta$) of chloroform values from 4 to 3988 ppb (or 69 ppb if three values above 69 ppb are excluded).

To arrive at the values reported in the table and figures, each of the 153 samples was analyzed in triplicate. Values for any one sample usually agreed within 15%, even when different instruments were used. A solution of known concentrations of seven

components: chloroform, methyl chloroform, trichloroethylene, bromodichloromethane, tetrachloroethylene, ethylene dichloride and chlorobenzene, was analyzed after every three analyses for use as an external reference for quantitation purposes. No other volatile halocarbon was detected above 1 ppb during this investigation. For those samples in which the $CHCl_3$ level exceeded 20 ppb, gas chromatographic–mass spectrometric (GC–MS) confirmation of the material was made based on the fragment ions appearing at 83, 85 and 87 atomic mass units.

*Expanded definition of circulating chloroform*

Throughout this discussion the term "circulating chloroform" has been used without comment. During analysis of blood plasma by this procedure[2], trichloroacetic acid (TCA) is thermally decomposed to yield $CHCl_3$, sometimes referred to as "derived chloroform". TCA results from the metabolism of trichloroethylene (3TCE) and tetrachloroethylene (4TCE), solvents used in dry-cleaning and degreasing industries, and is believed to be the primary source of derived $CHCl_3$.

Each participant chosen for this study was carefully screened; anyone with knowledge of exposure to tri- or tetrachloroethylene was not accepted. Other recognized sources of volatile halocarbons include: drinking water (trihalomethanes, 4TCE); soft drinks and reconstituted juices (trihalomethanes; 4TCE); margarine (chloroform, bromodichloromethane, trichloroethane and 3TCE); cigarette smoke (chloroform); and decaffeinated coffee (3TCE, 4TCE, methylene chloride). Cough syrup and dentifrice sources have been off the U.S. market for a number of years.

It must be emphasized that the participants in this study were encouraged to continue their usual lifestyles. Only individuals who would have had known vocational and/or recreational exposure to $CHCl_3$, 3TCE or 4TCE were excluded. The investigation was designed to study usual fluctuations and ranges of values within a group of subjects.

It turns out that plasma chloroform, no matter what the source, is one of the few reliable indices known to assess chronic exposure to volatile halogenated hydrocarbons. Apparently brominated analogues are too reactive, as mentioned earlier. In previously reported rodent work, Pfaffenberger *et al.*[5] observed comparatively little bromodichloromethane (BDCM) residue in the blood of rats dosed with relatively high amounts of BDCM, up to 5 mg/kg body weight for up to 25 days. Even when the animals were sacrificed 1 h after dosing, serum BDCM levels were low compared to chlorocarbon levels obtained for rats dosed with equivalent amounts of chlorinated volatile hydrocarbons and sacrificed after the same interval of time. The bulk of most ingested, absorbed or inhaled volatile organic compounds is rapidly eliminated via respiration. Apparently volatile halocarbons are not very highly sequestered by adipose tissue[2,5] as are the chlorinated pesticides and polychlorinated biphenyls.

*Future studies*

The results reported here comprise Phase I of a four-phase project. Fig. 6 presents hypothetical results similar to the type we anticipate obtaining over the next 18 months. During Phase II the only change in the sampling procedure will be a requirement that each participant ingests 15 fluid ounces of her usual drinking water 1 h prior to having her blood sampled. This may result in slightly higher averages for plasma $CHCl_3$ levels. The trihalomethane content of the ingested water will also be determined. A modest average increase of perhaps 10% is anticipated.

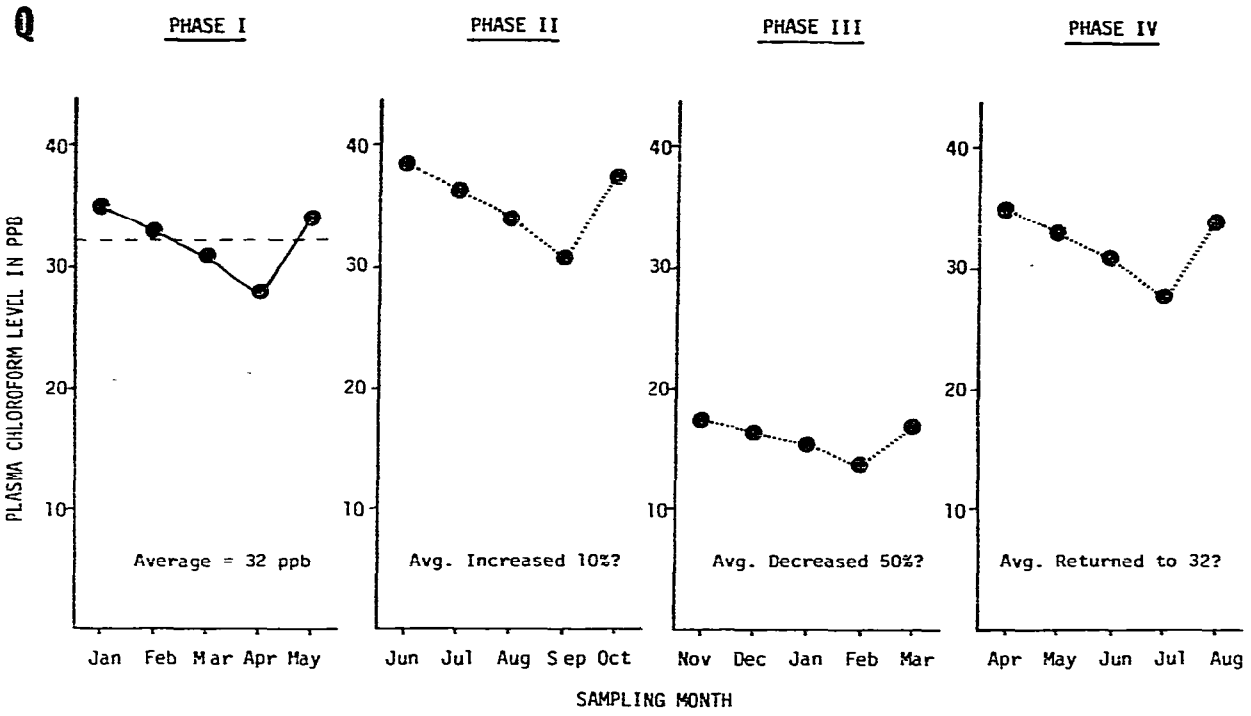



Fig. 6. Hypothetical results anticipated for subject Q during Phases II–IV of continued investigation. See Fig. 1 for further explanation and text for details.

During Phase III, the subjects will drink and cook with only pure bottled water containing no volatile halogenated organic compound. A significant average decrease of perhaps 50% is anticipated under these circumstances. Phase IV will be a repeat of Phase I to determine if initial average values for plasma chloroform levels are re-attained.

ACKNOWLEDGEMENTS

This paper is a small contribution to a rather large symposium held in honor of Professor Evan C. Horning. C.D.P. should like to recognize the generous and lasting contributions Dr. Horning has made to his continuing scientific career.

The authors also gratefully acknowledge the helpful advice of Leland J. McCabe, Frederick M. Kopfler and Robert D. Lingg; the GC–MS confirmatory analyses of Thomas V. Briggle; and the financial support of the U.S. Environmental Protection Agency through Copperative Agreement CR-807714-01.

REFERENCES

1 R. L. Jolley, H. Gorchev, D. H. Hamilton, W. A. Brungs, R. B. Cumming and V. A. Jacobs (Editors), *Water Chloration: Environmental Impact and Health Effects*, Vols. 1–3, Ann Arbor Sci. Publ., Ann Arbor, MI, 1975, 1977 and 1979.

2 A. J. Peoples, C. D. Pfaffenberger, T. M. Shafik and H. F. Enos, *Bull. Environ. Contamin. Toxicol.*, 23 (1979) 244.
3 T. A. Bellar and J. J. Lichtenberg, *J. Amer. Water Works Assoc.*, 66 (1974) 739.
4 C. D. Pfaffenberger, K. P. Cantor, A. J. Peoples and H. F. Enos in R. L. Jolley, W. A. Brungs, R. B. Cumming and V. A. Jacobs (Editors), *Water Chlorination: Environmental Impact and Health Effects*, Vol. 3, Ann Arbor Sci. Publ., Ann Arbor, MI, 1979, pp. 1059–1074.
5 C. D. Pfaffenberger, A. J. Peoples and H. F. Enos, in A. Zlatkis (Editor), *Advances in Chromatography 1979*, Chromatography Symposium, Houston, TX, 1979, pp. 639–652.