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BLOOD PLASMA LEVELS OF VOLATILE CHLORINATED SOLVENTS AND METABOLITES IN OCCUPATIONALLY EXPOSED WORKERS

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ABSTRACT

Blood samples from 157 employees of two metal-working plants which make extensive use of degreasing solvents like trichloroethylene (TRI), tetrachloroethylene (TETRA), and 1,1,1-trichloroethane (1,1,1-) were analyzed for solvent content as well as total chloroform ($CHCl_3$; underived plus derived) level. At one plant where an air contamination problem was suspected, 85% of the workers had trace (less than 1 ppb) to quantifiable amounts of one or more of the above mentioned solvents and total chloroform levels up to 2000 parts per billion (2 ppm). The average chloroform value for this group was 190 ppb, almost eight times the currently accepted value for the general population of Dade County, Florida. When the circulating $CHCl_3$ was divided into underived and derived components by a mass-spectrometric technique, it was determined that between 75% and 90% of the chloroform was being derived from a precursor, presumably trichloroacetic acid (TCA), a known metabolite of both trichloroethylene and tetrachloroethylene. A few residents living several miles from the plant of concern were sampled and found to have total chloroform levels similar to those found for many of the inhabitants of Dade County.

1. INTRODUCTION

Assessment of worker exposure to volatile chlorinated solvents by analysis of blood plasma can be complicated by a number of interrelated phenomena including: (a) accumulation of the lipophilic solvents in fatty tissue, possibly even brain, (b) considerably rapid excretion of inspired vapors by the lungs, (c) reactivity of the solvent with other constituents of the blood, (d) conversion of the parent compound (solvent) into corresponding metabolites, one or more of which may yield other chlorinated solvents as a result of thermal decomposition, and (e) various sampling and shipping parameters which may greatly affect the interpretive phases and statistical work-up of the studies.

Nevertheless, widespread exposure of workers to a large variety of chlorinated solvents has made it imperative for industrial hygienists and residue chemists to quantify blood-plasma levels of compounds such as chloroform ($CHCl_3$), trichloroethylene (TRI), tetrachloroethylene (TETRA) and 1,1,1-trichloroethane (1,1,1-). The latter three solvents are commonly used in degreasing and dry cleaning operations; in fact it was early work with a small group of dry cleaners that led to the studies discussed herein (1).

Among the dry cleaners examined, one who worked mainly in the room where garments were dipped into tetrachloroethylene had a total chloroform level of 6000 ppb with an accompanying TETRA value of 2300 ppb. A worker who spent only part of each day in the dip room exhibited 3000 ppb $CHCl_3$ and 800 ppb solvent. Two other cleaners who made occasional trips to the dip room and otherwise worked as spot cleaners had respective total chloroform/TETRA values of 350/100 ppb and 300/150 ppb.

The determination of blood-plasma levels of chlorocarbons is not always a straightforward analytical process. For example, both TRI and TETRA are converted by hepatic enzymes into trichloroacetic acid (TCA). Moreover, TCA is thermally decomposed into chloroform which is normally present in the circulating blood as a result result of eating and drinking foods and beverages (2) containing the trihalomethane or one or more of its precursors. In order to make the exposure assessments reported herein, it was necessary to employ a mass-spectrometric method by which underived $CHCl_3$ and thermally derived $CHCl_3$ (from TCA) could be differentiated.

2. OBJECTIVES OF RESEARCH

The primary purposes of this investigation were to:

a. Apply previously developed methodologies for determining human blood-plasma levels of volatile halogenated organic compounds to samples taken from occupationally exposed individuals.

b. Demonstrate whether or not blood levels of halogenated solvents fall during non-working periods (days off).

c. Compare blood levels of chlorinated solvents in workers of two different plants performing the same types of work but possessing different air conditioning systems.

d. Determine what blood-plasma levels of chlorinated solvents can be expected as a result of chronic exposure to air concentrations which rarely exceed 2% of the NIOSH recommended threshold limiting values (TLV's) suggested for the various volatile compounds.

e. Compare the worker levels with those of the surrounding citizens and values obtained for other non-occupationally exposed populations.

f. Differentiate in a sub-set of the samples how much of the chloroform as detected by the gas-chromatographic procedure can be considered present as $CHCl_3$ in the bloodstream and how much must be considered derived from trichloroacetic acid or another $CHCl_3$ precursor.

3. EXPERIMENTS

Blood samples were collected via venipuncture by qualified medical personnel and stored under refrigeration pending analysis according to published protocol (3). Quantification of blood-plasma levels of chloroform and other chlorocarbons was aecomplished by the gas-chromatographic procedure described in 3.1 below. Differentiation between derived (from TCA via TRI and TETRA) and underived $CHCl_3$ was according to the mass-spectrometric method given in 3.2. Solvent 1,1,1-trichloroethane did not yield trichloroacetic acid or chloroform from either metabolism or thermal decomposition.

3.1 Gas-Chromatographic Quantification Procedure

Quantification of the chlorocarbon solvents present in the blood samples was accomplished by following the published gas-chromatographic procedure (3) of Peoples, Pfaffenberger, et al. As discussed in the above-cited article, the method, which was based on the earlier purge/trap/desorption methodology of Bellar and Lichtenberg (4), readily allowed quantification to one part of solvent per billion parts of blood serum (1 ppb), with a standard deviation of ± 15%. During collaborative work with Pierson and coworkers (5) of the Research Triangle Institute, Research Triangle Park, it was established that the variance of duplicate specimen analyses by this method was 5.76 with a coefficient of variation of 17.7%. Moreoever, replicate analyses over time of a blood specimen pool indicated that no degradation (lowering) of the chloroform value occurred during a 10-week storage interval.

The analytical procedure involved heating the specimen in the presence of an antifoaming agent while purging the volatile organic chemicals from the solution using a flow of inert gas, usually nitrogen. The purged compounds were directed to an adsorbent trap of Tenax GC. After completion of the purge/trap period, volatile analytes were thermally desorbed from the adsorbent trap to a gas-chromatographic column which was temperature programmed to maximize resolution of the various components. This procedure is applicable to the quantification of over 25 volatile halocarbons, but during this investigation only 11 were of interest: chloroform ($CHCl_3$); ethylenedichloride (DCE); 1,1,1-trichloroethane (1,1,1-); carbon tetrachloride (CCl_4); bromodichloromethane (BDCM); trichloroethylene (TCE); dibromochloromethane (DBCM); bromoform ($CHBr_3$); tetrachloroethylene (TETRA); chlorobenzene (ClBz); and vinylidene chloride (VCD). Inasmuch as only $CHCl_3$, TRI, TETRA and 1,1,1- were detected during these studies, zero levels of the other 7 compounds were not included in Tables 1 - 5. Other volatile organic compounds which may be quantified by this procedure include: methylene chloride, cis-1,2-dichloroethylene, trans-1,2,-dichloroethylene, ethylidene chloride, chlorobromomethane, 2,3-dichloro-1-propene, cis-1,3-dichloro-1-propene, trans-1,3-dichloro-1-propene, ethylene dibromide (EDB), 1,1,1,2-tetrachloroethane, 1-chlorohexane, acetylene tetrachloride, pentachloroethane, 3-chloro-1-bromopropane, hexachloro-1,3-butadiene and 1,4-dichlorobutane.

3.2 Mass-Spectrometric Differentiation Procedure

To differentiate between circulating (underived) $CHCl_3$ and circulating trichloroacetic acid (TCA) in the bloodstream of workers exposed to TCA, TRI and/or TETRA, the blood plasma was thermally decomposed in the presence of 80% D_2O (6). Under the experimental conditions employed, the decomposition of TCA passed through the formation of the trichloromethyl carbanion, CCl_3^-, which abstracted a deuterium atom 80% of the time to form $CDCl_3$. In 20% of the extractions, $CHCl_3$ formed via proton extraction from water, so a 20% correction was necessary to better indicate the amount of underived chloroform. (The plasma sample was not freeze-dried because the underived chloroform would have been lost during this step of the analytical procedure.)

Within the mass spectrometer, $CDCl_3$ molecules gave rise to a major positively charged fragment, $CDCl_2^+$, with m/z = 84. The major corresponding ion ($CHCl_2^+$) from $CHCl_3$ occurred at m/z = 83. From the relative abundances of the two ions (plus the aforementioned 20% correction for the fragment at m/z = 83), the amount of derived chloroform (from TRI and TETRA) was calculated (6). Shifts ranged from 75% to 90% as discussed below.

4. ANALYTICAL RESULTS

Analyses of samples have been summarized in Tables 1 - 6.

Table 1. Blood Plasma Chlorocarbon Levels (ppb) of Some Office Personnel Working at Plant A

SAMPLE	$CHCl_3$	TRI	TETRA	1,1,1-
1-a	147	T *	3	T
1-b	175	T	7	T
2-a	150	T	3	0 **
2-b	400	T	3	T
3-a	366	T	T	22
3-f	300	T	6	T
3-m	250	T	7	T
4-a	270	0	5	0
4-f	600	T	2	T
4-m	420	T	5	T
5-a	463	0	3	0
5-f	800	T	9	T
5-m	720	T	7	T
6-a	207	0	9	0
6-f	400	T	2	T
6-m	290	T	6	T
7-a	252	0	5	0
7-f	520	T	5	T
7-m	500	T	2	T
8-a	282	0	5	0
8-f	790	T	8	T
8-m	460	T	3	T
9-a	100	0	3	20
9-f	285	T	4	T
9-m	350	T	1	T
10-a	235	T	3	0
10-c	25	0	0	0

* A value of T signifies a trace < 1 ppb detected
** A value of 0 signifies not a trace was detected

Table 2. Blood Plasma Chlorocarbon Levels (ppb) of a Small Group of Workers in Plant A

SAMPLE	$CHCl_3$	TRI	TETRA	1,1,1-
1	48	3	0 *	3
2	36	3	0	3
3	10	0	0	0
4	13	0	0	0
5	100	5	0	2
6	110	2	0	2
7	18	3	0	2
8	38	2	0	2
9	43	3	0	3
10	9	0	0	2
11	36	3	12	6
12	19	0	0	T **
13	19	0	0	2
14	75	2	0	3
15	370	4	0	4
16	18	0	0	2
17	13	0	0	3
18	100	4	0	3
19	190	2	0	4
20	140	2	0	2
21	880	5	2	0
22	200	0	T	3
23	530	T	3	0
24	500	0	3	7
25-a	340	4	0	2
25-b	410	3	0	3
26-a	950	10	0	23
26-b	1,150	12	0	19
27-a	1,885	4	0	0
27-b	1,965	7	0	0

* A value of 0 signifies not a trace was detected
** A value of T signifies a trace < 1 ppb detected

For this investigation the gas-chromatographic (GC) method of Peoples, Pfaffenberger and coworkers (3) was applied to 187 blood samples, often run in duplicate or triplicate; and the mass-spectrometric (GC/MS/DS) procedure of Pfaffenberger, Briggle and Peoples (6) was utilized for 21 analyses. The methods appear applicable to a wide range of concentrations of solvent residues. When a very high level of solvent is discovered in a particular sample, the sample volume is conveniently adjusted downward. Most of the blood samples were obtained from people who were working around degreasing operations. Blood levels of chloroform, trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane were anticipated. It should be recognized that in no instance were Plants A and B violating NIOSH recommended TLV values for the latter 3 solvents. In fact, according to air monitoring records, neither plant ever exceeded 2% of the recommended TLV's for any of the solvents; so the findings

Table 3. Blood Plasma Chlorocarbon Levels (ppb) of a Group of Workers at Plant B

SAMPLE	$CHCl_3$	TRI	TETRA	1,1,1-
1	10	0 *	0	0
2	30	0	0	0
3	5	0	0	0
4	15	0	0	0
5	64	0	0	0
6	11	0	0	0
7	8	0	0	0
8	15	0	0	0
9	10	0	0	0
10	10	0	0	0
11	7	0	0	0
12	52	0	0	0
13	21	0	0	0
14	13	0	0	0
15	13	0	0	0
16	7	0	0	0
17	31	0	0	0
18	7	0	0	0
19	8	0	0	0
20	5	0	0	0
21	10	0	0	0
22	14	0	0	0
23	10	0	0	0
24	14	0	0	0
25	45	0	2	4
26	52	0	2	0
27	47	0	0	2
28	22	0	0	0
29	36	0	0	0
30	6	0	0	0
31	8	0	0	0
32	138	0	2	5
33	72	0	9	16
34	61	0	4	7
35	40	0	0	10
36	550	0	17	18
37	155	0	5	22
38	32	0	4	2
39	6	0	0	3
40	78	0	7	6

* A value of 0 signifies not a trace was detected

of the investigation fall within the domain of currently acceptable blood-plasma levels of solvents (solventemia) resulting from chronic exposure to volatile chemicals in a workplace setting.

Table 1 summarizes the first analyses performed on workers located at Plant A. Samples 1-a, 1-b, 2-a, 2-b, 3-a, 4-a, 5-a, 6-a, 7-a, 8-a, and 9-a were collected from office personnel not located within the plant but in a room adjacent to the facility and utilizing the same air-conditioning system.

Table 4. Blood Plasma Chlorocarbon Levels (ppb) of a Large Group of Workers of Plant A

SAMPLE	$CHCl_3$	TRI	TETRA	1,1,1-
1	48	3	0 *	3
2	36	3	0	3
3	10	0	0	0
4	13	0	0	0
5	100	5	0	2
6	110	2	0	2
7	18	3	0	2
8	38	2	0	2
9	43	3	0	3
10	9	0	0	2
11	36	3	12	6
12	19	0	0	T **
13	19	0	0	2
14	75	2	0	3
15	370	4	0	4
16	18	0	0	2
17	13	0	0	3
18	100	4	0	3
19	190	2	0	4
20	140	2	0	2
21	30	2	0	2
22	12	0	0	1
23	100	1	0	4
24	1,500	7	0	6
25	300	3	0	2
26	1,000	15	0	0
27	200	2	3	3
28	22	1	0	2
29	20	2	0	2
30	11	3	0	3
31	12	2	0	2
32	46	3	0	4
33	800	4	0	2
34	310	0	0	7
35	18	2	0	2
36	70	0	0	1
37	10	2	0	2
38	275	0	0	6
39	58	4	0	1
40	250	0	0	3
41	18	1	0	2
42	1,600	15	0	8
43	26	3	0	3
44	400	4	0	2
45	410	3	0	4
46	33	3	0	4
47	95	3	0	0
48	105	0	0	2
49	500	3	0	6
50	28	1	0	3

* A value of 0 signifies not a trace was detected
** A value of T signifies a trace < 1 ppb detected

Table 4. Blood Plasma Chlorocarbon Levels (ppb) of a Large Group of Workers of Plant A (continued)

SAMPLE	$CHCl_3$	TRI	TETRA	1,1,1-
51	500	0 *	0	4
52	35	1	0	2
53	30	1	0	1
54	305	0	0	3
55	44	0	0	0
56	17	4	0	0
57	68	2	0	0
58	190	1	8	2
59	35	2	11	7
60	225	T **	0	2
61	17	0	0	0
62	57	0	0	0
63	15	0	0	1
64	12	0	0	0
65	47	3	2	0
66	18	1	0	0
67	38	2	0	0
68	2,000	22	8	14
69	390	6	0	3
70	16	1	0	0
71	12	3	0	0
72	100	3	0	2
73	11	3	0	0
74	10	4	0	0
75	225	4	0	0
76	25	3	0	0
77	325	3	0	1
78	30	3	0	3
79	515	3	0	5
80	225	1	0	2

* A value of 0 signifies not a trace was detected
** A value of T signifies a trace < 1 ppb detected

Traces to quantifiable amounts of TRI, TETRA, and 1,1,1- were found in all of the participants; and values for total $CHCl_3$ ranged from 100 to 463 ppb. Inasmuch as these levels were considerably higher than any found during several years of study of the general population in and around Dade County, Florida (7), 7 of the particpants agreed to be re-sampled on both Friday at the end of the workweek (3-f through 9-f) and again on the following Monday (3-m through 9-m) before returning to work. For 6 of the 7 participants, the blood-plasma level of $CHCl_3$ fell between 4 and 42 percent. Participant 10 went on a 2-week vacation to a pristine (clean air) environment after donating sample 10-a. At the end of his vacation his $CHCl_3$ residue level had fallen from 235 ppb to 25 ppb, the generally accepted average for our studies centered around Dade County, Florida (7).

Sampling continued at Plant A (Table 2). This group consisted of 20 randomly chosen participants (samples 1 - 20), 4 workers who had been previously studied (samples 21 - 24), and pregnant workers who were sampled twice (samples 25-a - 27-b). Traces to quantifiable amounts of TRI, TETRA, and 1,1,1- were

Table 5. Blood-Plasma Chlorocarbon Levels (ppb) of Citizens Non-Occupationally Exposed to Chlorinated Solvents But Residing Only a Few Miles Away From Plant A

SAMPLE	$CHCl_3$	TRI	TETRA	1,1,1-
1	10	0 *	0	0
2	12	0	0	0
3	53	0	0	0
4	26	0	0	0
5	7	0	0	0
6	28	0	0	0
7	16	0	0	0
8	18	0	0	0
9	9	0	0	0
10	20	0	0	0

* A value of 0 signifies not a trace was detected

Table 6. Percent Derived and Underived Blood-Plasma Chloroform in Selected Samples as Determined by a Mass Spectrometric Method

SAMPLE	DERIVED FROM SOLVENTS	UNDERIVED (FROM DIET)
1-6-f *	90 %	10 %
1-9-f	87 %	13 %
2-25-a	85 %	15 %
2-27-a	89 %	11 %
4-01	79 %	21 %
4-09	80 %	20 %
4-15	88 %	12 %
4-21	82 %	18 %
4-24	88 %	12 %
4-26	88 %	12 %
4-27	90 %	10 %
4-32	78 %	22 %
4-33	88 %	12 %
4-34	89 %	11 %
4-39	75 %	25 %
4-46	78 %	22 %
4-49	89 %	11 %
4-52	78 %	22 %
4-55	79 %	21 %
4-62	80 %	20 %
4-67	77 %	23 %
** Range:	75 - 90 %	10 - 25 %
Average:	83 %	17 %

* First number refers to previous tables; rest of numerals and letters refer to samples within the table indicated

** Range and average data calculated for Table 4 values only

found in 93% of this group. The 4 office personnel had $CHCl_3$ values ranging from 200 ppb to 880 ppb, and the 3 pregnant office workers had respective average $CHCl_3$ levels of 375, 1,050 and 1,925 ppb. Exposure appeared related to air intake of the air conditioning system.

Samples were next collected from another location, Plant B (Table 3). Only 30% of the 40 randomly sampled workers had quantifiable levels of TETRA and/or 1,1,1- in their bloodstream. No trichloroethylene was detected; and values for total chloroform ranged from 5 ppb to 550 ppb.

The data from Plant B workers suggested that there was a problem peculiar to Plant A. An additional 80 samples were randomly collected from individuals falling into most of the possible job categories of Plant A. Trace to quantifiable amounts of TRI, TETRA, and 1,1,1- were found in 85% of this group. Total chloroform values ranged from 9 ppb to 2,000 ppb with an average value of 190 ppb. In 81% of the samples containing 100 ppb or more $CHCl_3$, either TRI or TETRA or both were also detected during the same analyses. The average $CHCl_3$ value for the 15% of the workers who had no other chlorinated solvent in their blood was 26 ppb, a level essentially equal to the amount we generally regard as average for citizens of Dade County (7).

Ten citizens who lived several miles from Plant A but were not employed there were sampled and analyzed for comparison purposes. As indicated in Table 5, no one in this group had TRI, TETRA, or 1,1,1- in his bloodstream. The average chloroform level for this group was 20 ppb, 20% lower than the accepted value for the general population of Dade County (7).

GC/MS/DS methodology (6) was applied to 21 of the samples of Plant A workers. Table 6 cross-references these samples to values given in Tables 1, 2, and 4. Thus, sample 1-6-f in Table 6 is sample 6-f of Table 1; sample 2-27-a is sample 27-a of Table 2; and sample 4-27 is sample 27 of Table 4.

Samples analyzed according to the GC/MS/DS procedure were from workers known to be exposed to either trichloroethylene, tetrachloroethylene, or both. On a percentage basis, between 10% and 25% of the chloroform value was underived from a trichloromethyl carbanion precursor and probably was present as a result of dietary consumption. Between 90% and 75% of the chloroform was apparently derived from some precursor, presumably trichloroacetic acid. This suggested than an average of 83% of the body burden of chloroform in blood plasma was related to occupational exposure, most probably via inhalation. It was strongly suggested that the air-conditioning intake be far removed from the solvent vapors constantly present in the main portion of Plant A, but no further sampling was possible.

5. CONCLUSIONS

Employees working around degreasing solvents such as trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane often have quantifiable amounts of solvents in their blood even when the air concentrations of these volatile organic compounds do not exceed federally imposed maximum levels. In certain circumstances, office personnel in close proximity to solvent use may also exhibit solventemia resulting from chronic exposure to organic vapors. Every effort must be made to assure that fresh air intake is from an uncontaminated air supply. By use of gaschromatographic techniques, blood-plasma solvent levels up to several thousand parts per billion may be detected in chronically exposed workers. Mass-spectrometric methodology utilizing deuterium oxide allows differentiation between the derived and underived chloroform. Total blood-plasma

$CHCl_3$ levels in chronically exposed workers may be eighty times higher than those of the general population, but the resulting health effects are unknown.

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