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Correlation of Tetrachloroethylene in Blood and in Drinking Water: A Case of Well Water Pollution

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Tetrachloroethylene (to be abbreviated as TETRA), an established animal carcinogen (National Cancer Institute 1977; National Toxicology Program 1986; Mennear et al. 1986), has been widely used for the cleaning of cloth for many years (Browning 1965). Currently, this chemical together with trichloroethylene (TRI) appears to be the most prevailing pollutant of ground water (Zoeteman et al. 1980; US Council of Environmental Quality 1982; Environment Agency 1983; Trouwborst 1983) in various countries including Japan. While the biological monitoring of TETRA exposure is popular in occupational health (for a review, see Monster and Zielhuis 1983), TETRA was seldomly analyzed in the subjects exposed through the general environment. In the present report, a case of water pollution with TETRA is described in which up to 5 μg/L TETRA was detected in the blood of the inhabitants in a area where well water was contaminated with TETRA.

MATERIALS AND METHODS

Inhabitants in a area downstream (in a flow of a brook and possibly also that of ground water) of a dry-cleaning plant complained that their well water had "chemical" smell especially when it was boiled. Some 36 families (about 140 people) appeared to be involved, among which treated water (city water) had been supplied by the local authority to 23 homes, but not to the remaining 13. Interview disclosed that the inhabitants including those who had treated water supply depended primarily on well water for daily life and preferred well water even for drinking and cooking; they disliked the treated water because of its "chlorine" smell due to chlorination. The treated

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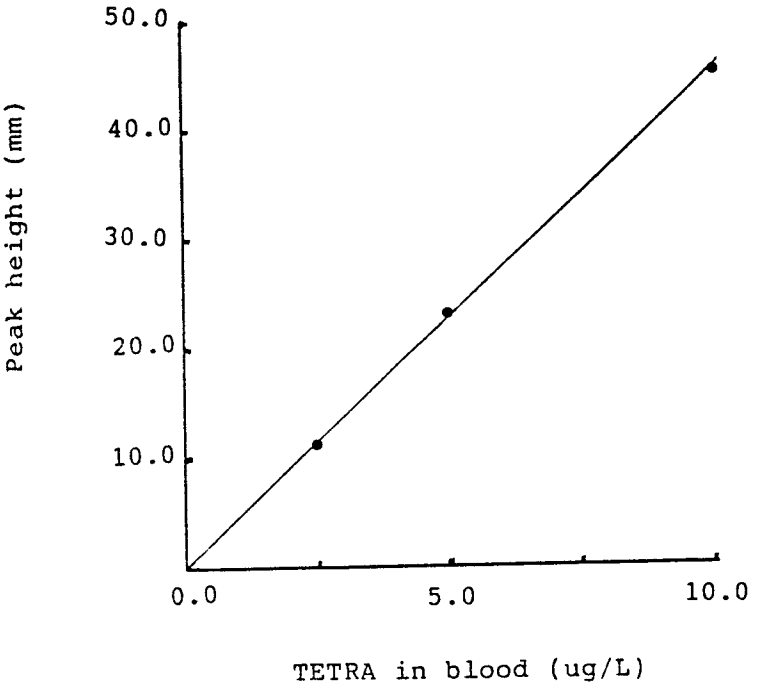

Figure 1. A calibration line for head-space GC determination of TETRA in blood.

water was obtained by rapid filtration of river water followed by chlorination, and no TETRA nor TRI had ever been detected in the treated water. Venous blood samples were collected from 74 members of 23 families together with water samples from the wells (all <10 m-deep) of 34 homes including those of the 23 families. From each blood samples a few mL portion was taken in a plastic container with no air space, sealed, kept frozen and analyzed immediately after thawing, and the rest was used for clinical laboratory tests. Well water samples were taken in 200-mL containers with no air space, kept at 4 °C in dark and analyzed within 24 hrs after sampling.

An ECD(^{63}Ni)-gaschromatograph (GC: Hitachi Model 163) was employed for the detection of TETRA both in blood and in water. The GC was equipped with a glass column (3 m in length and 3 mm in inner diameter) packed with 20% Silicon DC 550 on Chromosorb WAW DMCS (60-80 mesh). The injection port and the oven were heated at 150°C and 90°C, respectively. Nitrogen gas, a carrier, was allowed to flow at a rate of 50 mL/min.

TETRA in blood was determined by head-space GC

(Ministry of International Trade and Industries 1987). In practice, 2 mL of blood was taken in a 10-mL vial [with a silicone septum (AK 12024: Gasukuro Kogyo Co., Tokyo, Japan)]. The vial was sealed immediately after the addition of 0.5 g NaCl and 5 µL methanol, heated at 25°C (range; ±0.1 °C) in a water bath for 1 hr. An aliquot, 0.25 mL, of the air phase was injected to the GC by means of an air-tight syringe. The calibration line (Fig. 1) was prepared by the addition of 5 µL each of TETRA in methanol (1 to 100 µg/mL methanol) to 6 blood sample obtained from non-exposed control subjects. The lines passed the origin and the CV of the slopes was ca. 10% when the calibration line setting was repeated 6 times. The lowest limit of determination was 0.5 µg TETRA/L blood when a peak/noise ratio of 2 was taken. Analysis of water for TETRA was conducted in accordance with the standardized method (Ministry of International Trade and Industries 1987). Namely, TETRA in 40 mL of sample water was extracted with 10 mL n-hexane by vigorous shaking for 15 min, and 2-5 µL of the organic layer (diluted with n-hexane as necessary) was applied to the ECD-GC analysis. TRI was also measured when the level was above the detection limit. The lowest limit of determination for TETRA and TRI in water were both 0.2 µg/L.

Serum biochemistry [assay for GOT (ASAT; EC 2.6.1.1), GPT (ALAT; EC 2.6.1.2), gamma-GTP (EC 2.3.2.1), ZTT, TTT and serum protein] and hematology (hemoglobin and hematocrit) were conducted by conventional methods.

RESULTS AND DISCUSSION

When water samples were collected from wells in 34 households for TETRA determination and the results were plotted on a map, a marked clustering of high TETRA values was observed along a flow of a brook (Fig. 2), whereas TETRA levels were much lower in the samples collected at the sites away from the brook. Upstream of the sites with high TETRA values was a factory suspected of the water pollution source. The factory used TETRA for dry-cleaning and TETRA ater use was stored in a semi-underground tank. At the points further upstream, TETRA was not detected in well water samples as expected. TRI was also found in most of well water samples in which TETRA was detected. When TRI/TETRA ratios were calculated for each sample and correlation of the ratio (Y%) with the distance (X m) from the suspected point source was estimated as the best fit (by least square method) for an equation of $\log Y = \alpha \times \log X + \beta$, $\alpha=1.167$ ($r=0.507$; $p=0.05$) was obtained as the solution. The equation indicates that

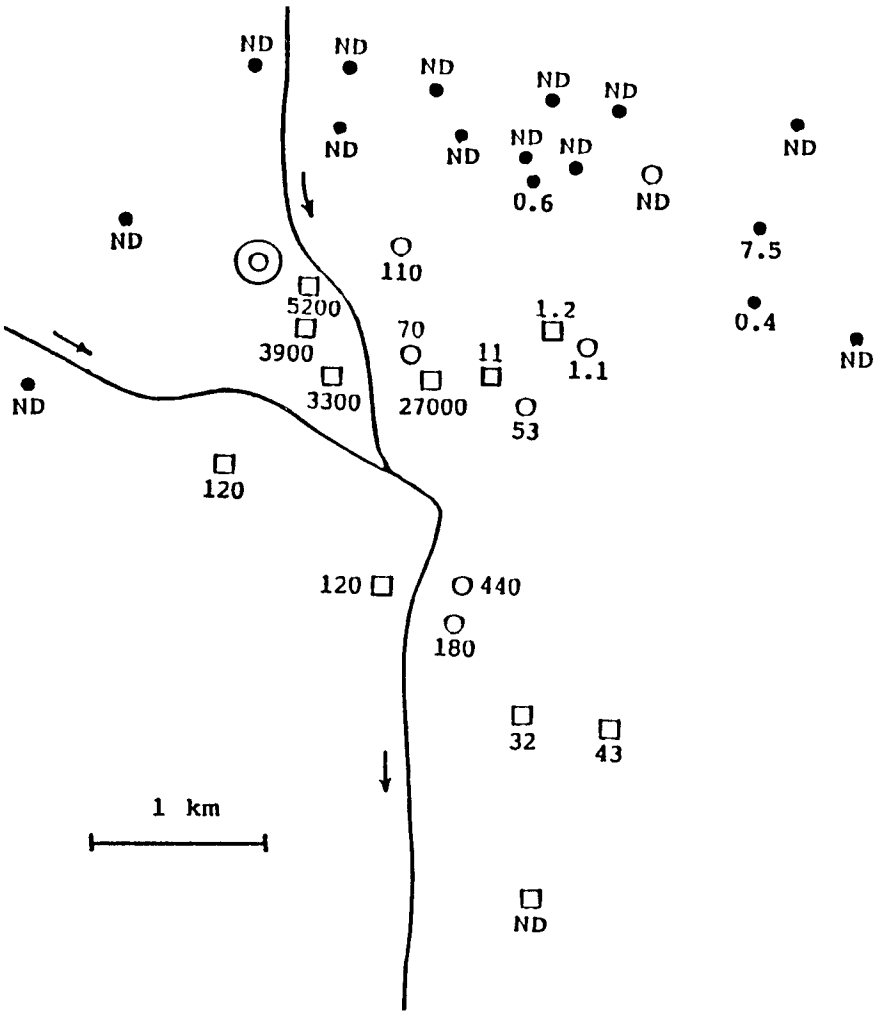

Figure 2. TETRA levels in wells.
The double circle indicates the location of probable point source of pollution. Other symbols show the locations of wells surveyed, and the numbers are TETRA levels (μg/L; ND <0.2 μg/L). Open squares and circles are for the wells of the families who offered blood samples (squares for those who had no treated water supply, and circles for those who used both well water and treated water). Solid circles show the cases in which only well water (and no blood) samples were available. The curve means a tiny brook flowing through the study region.

Table 1. TETRA in blood and in well water by family

Family number (No.[a])	TETRA (μg/L) in Well water	TETRA (μg/L) in Blood	Use of well water for D/C[b]	Supply of treated water
1 (5)	27000	5.1, 4.6, 3.8, 1.5, 1.0	yes	no
2 (2)	5200	2.2, 0.8	yes	no
3 (1)	3900	4.3	yes	no
4 (2)	3300	0.9, ND[c]	yes	no
5 (5)	440	ND, ND, ND, ND, ND	no	yes
6 (5)	180	ND, ND, ND, ND, ND	yes	yes
7 (6)	120	0.7, ND, ND, ND, ND, ND	yes	no
8 (5)	120	ND, ND, ND, ND, ND	yes	no
9 (4)	110	ND, ND, ND, ND	yes	yes
10 (1)	70	ND	yes	yes
11 (3)	53	ND, ND, ND	yes	yes
12 (5)	43	ND, ND, ND, ND, ND	yes	no
13 (5)	32	ND, ND, ND, ND, ND	yes	no
14 (4)	11	ND, ND, ND, ND	yes	no
15 (2)	1.2	ND, ND	yes	no
16 (1)	1.1	ND	yes	yes
17 (1)	ND	ND	yes	no
18 (4)	ND	ND, ND, ND, ND	yes	yes

In addition, TETRA levels in blood were ND in 13 subjects of 5 families who had no well.
a/ Number of examinees.
b/ For drinking and cooking.
c/ Not detectable (<0.2 μg/L for well water and <0.5 μg/L for blood).

the TRI/TETRA ratio will increase as almost as a linear function of the distance from the source.

Among the 74 individuals (of 31 families) who offered blood samples, 5 families with 13 subjects had no well and possibilities of TETRA exposure via well water could be ruled out; no TETRA was detected in their blood as expected. Results with the remaining 18 families (with 61 subjects) are summarized in Table 1 together with information on use of well water/treated water for drinking and cooking. It was evident from the table that TETRA was detected in the blood in case TETRA in well water was above a certain level, e.g., 120 μg/L, whereas TETRA was not detectable in blood either when TETRA in water was very low (e.g., <1 μg/L) or well water was not used for drinking or cooking. For example, TETRA in blood was below detection limit in the case of Family No. 5 despite that TETRA was present at 440 μg/L in their well water, presumably because they depended only on

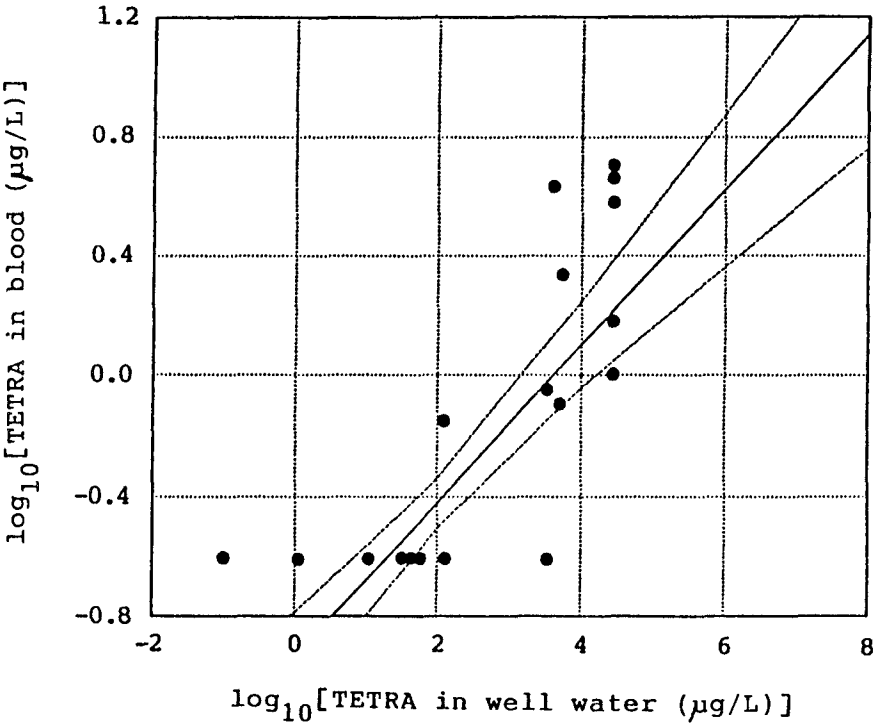

Figure 3. The correlation between TETRA in well water and TETRA in blood of the family who used the well water for drinking and cooking.

The dots indicate 38 cases of the examinees who had no treated water supply, and offered both blood and well water samples (i.e., members of Family Nos. 1-4, 7, 8, 12-15, and 17 in Table 1). The line in the center is a calculated regression line of Y=AX+B, where Y and X are $\log_{10}$[TETRA (μg/L) in well water] and $\log_{10}$[TETRA (μg/L) in blood]. The dotted curves on both sides of the line are 95% confidence range of the regression line. TETRA levels in ND cases are taken as if they were half the detection limit, i.e., 0.1 μg/L and 0.25 μg/L for TETRA in well water and blood, respectively, in calculation for practical reasons.

treated water. Reversely, one member in Family No. 7 had 0.7 μg/L TETRA in blood probably the family used well water for life although TETRA in well water (120 μg/L) was lower than that for Family No. 5.

Trials were made to examine if there existed any correlation between TETRA in well water and TETRA in blood of the family who used the well water, and to figure out critical TETRA concentration in well water for detectable level of TETRA in blood in case the

correlation was present. For this purpose, families were selected so that TETRA was detectable in blood of at least one family member, and regression analysis was conducted between TETRA in well water and TETRA in blood, taking ND in well water or in blood as if it were 0.1 μg/L for the former or 0.25 μg/L for the latter (or half the detection limit) for practical reasons. The results are depicted in Fig. 3 in which the calculated regression line is drawn in a scatter diagram of TETRA in blood against TETRA in well water. A regression of $Y = 0.269X + 0.948$ was figured out in which X is $\log_{10}$[TETRA in well water (μg/L)] and Y is $\log_{10}$[TETRA in blood (μg/L)]. When TETRA in blood is assumed to be at the detection limit of 0.5 ug/L (or half the level, i.e., 0.25 μg/L), corresponding TETRA in well water is 287 μg/L (or 20 μg/L).

There was no case of abnormal hematology among the subjects who offered the blood. As for the liver functions, three men had elevated gamma-GTP levels (78, 163 and 188 units/mL) with normal GOT and GPT, and one woman had slightly elevated GOT (46 Karmen units) and GPT (65 Karmen units). TETRA levels in blood was below the detection limit in all of them.

While TETRA contamination of ground water with the risk of drinking pollution has been discussed since 1970's (e.g., Giger and Molnar-Kubica 1978; Page 1981), no trial appears to be made to detect the pollutant in human biological specimens. In the present study, TETRA was successfully detected in the blood of the inhabitants who used to use TETRA-contaminated well water for drinking and cooking (Table 1). There was a linear relation between TETRA levels in well water and TETRA in blood (Fig. 3). The critical TETRA concentration in well water for the detection of TETRA in blood (0.5 μg/L) was calculated to be 200 μg/L (Fig. 3). It should be noted however that the regression line obtained is subject to vary depending on the evaluation of ND values; the current calculation was based on the assumption of 0.25 μg/L in the place of ND, but the slope of the regression line will be less steep and thus the critical concentration could be smaller when smaller values (e.g., 0.1 μg/L) are assumed for ND values in blood. When the families were selected so that at least one family member had measurable level of TETRA (i.e., Families Nos. 1-4 and 7 in Table 1), the regression analysis made only with these subjects gave a critical concentration of 85 μg/L well water.

For practical solution of the case, treated water was supplied to all homes in the region and inhabitants were advised not to use well water for drinking and

other exposure-related purposes. The storage tank for used TETRA was repaired to prevent any leakage.

No TETRA exposure-related health effect could be demonstrated in the present study. An occupational exposure limit of 50 ppm (269 mg/m^3) is generally accepted for TETRA in many countries (e.g., Japan Association of Industrial Health 1988; American Conference of Governmental Industrial Hygienists 1988-1989). With assumptions that the respiratory volume is 0.9 m^3/hr and lung absorption rate for TETRA is 50% (Ohtsuki et al. 1983), 8 hr exposure to TETRA at 50 ppm will result in the absorption of 1206 mg TETRA. The highest TETRA level in the well water observed in the present study was 27 mg/L (Table 1). A daily intake of 2 L of such water will be associated with the ingestion of 54 mg TETRA. The amount to be absorbed would be even less because 100% absorption of TETRA in the gastro-intestinal tract is quite unlikely. Such a large difference in the amount to be absorbed may explain apparent lack of health effects in the present observation. The additional exposure of inhabitants to TETRA might have occured either through inhalation or even via skin penetration when the contaminated well water was heated and employed for bathing. There was however no measure to evaluate the intensity of such exposures.

Another point of interest is the co-presence of TRI in TETRA-contaminated well water. Several possibilities can be proposed. TETRA used in the suspected facility may originally contain TRI as an impurity, or TRI may be derived from other sources. In this relation, it should be noted that there was no other industrial plant in the region. While no sample was available to examine the first hypothesis, the facts that TRI was co-present in all well water samples and that the TRI/TETRA ratio was similar in two well water samples of highest TETRA levels might be in the favor of this possibility. The observation that the TRI/TETRA ratio increased as a linear function of the distance from the suspected pollution source however suggests that the ratio in well water increases as the contaminated water flows through soil. In fact, transformation of TETRA to TRI under reductive conditions has been recently confirmed at an experimental level (Bouwer and McCarty 1983; Vogel and McCarty 1985). It is also possible that TETRA may be selectively adsorbed to soil. The mechanism of TRI co-presence apparently needs further study.

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