



# Dynamics of Excretion of Thiodiacetic Acid into Urine in Polyvinyl Chloride Production Workers

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## Abstract

**Background:** Thiodiacetic acid (TDAA) is the main metabolite of vinyl chloride (VC) and 1,2-dichloroethane (EDC) and its urinary level is correlated with the level of exposure to these chemicals.

**Objective:** To study dynamics of the excretion of TDAA into urine of polyvinyl chloride (PVC) production workers.

**Methods:** The study sample consisted of 65 workers of VC and PVC divisions with various time intervals following exposure to the chemicals, 10 shift workers from PVC division, and 34 workers not exposed to the chemicals (control group). Analysis of urinary TDAA was carried out with gas chromatography with mass-selective detector.

**Results:** The concentrations of TDAA in the urine of workers of the VC division and in group of primary occupations who had a high level of exposure to the chemicals, were significantly ( $p < 0.05$ ) higher than that of workers of the PVC production division and group of auxiliary professions. The highest levels of TDAA in the urine of workers were found at the beginning of the next shift and during a long break, 24–48 hours after the cessation of the exposure.

**Conclusion:** When conducting biomonitoring studies in PVC production workers, the optimal time for collecting urine samples is at the beginning of the next shift or during a long rest, 24–48 hours after the exposure.

**Keywords:** Vinyl chloride; thiodiacetic acid [Supplementary Concept]; Environmental monitoring

## Introduction

Employees of modern large scale production plants of polyvinyl chloride (PVC) experience prolonged exposure to relatively low concentrations of chemicals, predominantly vinyl chloride monomer (VC) and 1,2-dichloroethane (EDC).<sup>1,2</sup> These pollutants are polytropic toxicants having mutagenic, carcinogenic,

hepatotropic, psychotropic, and irritating effects on the body.<sup>3–6</sup> Therefore, identification and measurement of the concentration of these toxicants or their metabolites in biological fluids (eg, blood and urine) to assess the degree of internal exposure and harmful effects they might exert are of paramount importance.

It has been established that VC and EDC entering the body are transformed

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predominantly by liver enzymes, forming reactive metabolites. The main reactive metabolites are 2-chloroethylene oxide, monochloroacetic acid, and conjugated metabolic product of thiodiglycolic (thio-diacetic) acid (TDAA). VC enters the body quickly until it reaches a concentration in the blood in equilibrium with the concentration of the inhaled VC.<sup>7</sup> Therefore, the net dose of the absorbed VC may be less than its intake into the body, especially at high concentrations, leading to saturation of metabolic enzymes.<sup>8</sup>

Previous studies have shown that TDAA is the main metabolite of VC and EDC, and can be used as a biomarker to assess their associated effects.<sup>9,10</sup> It was found that workers exposed to VC had a dose-dependent excretion of TDAA into their urine. It was also noted that the level of TDAA in urine is best detected at the beginning of the next work shift. Therefore, to assess the risk of exposure to these organochlorine compounds on the body, it is important to carry out biological monitoring at the workplace and determine the dynamics of excretion of TDAA in urine in exposed workers. We therefore conducted this study to determine the level of excretion of TDAA in the urine of workers working in the primary occupations of PVC production in different work shift schedules and at various times after the cessation of the exposure.

## Materials and Methods

The study groups included 75 men working at a large PVC plant in Siberia, Russia. All research participants were employees of VC and PVC divisions, and classified as either workers in primary occupations (*ie*, gas separation and polymerization operators, and tank cleaners) and auxiliary professions (*ie*, mechanics, instrumentation mechanics, and shift managers). During their shift, operators inspect the

production equipment, and monitor the technological process both in the shops and remotely from the control room. Tank cleaners periodically clean the pipelines and the equipment to remove PVC resin. Mechanics and shift managers work both directly in the shops and in the office space.

In our previous study,<sup>1,2</sup> it was shown that workers of the VC division were subjected to simultaneous intensive exposure of concentrations of VC ranging from 2.0 to 14.6 mg/m<sup>3</sup> and EDC in the range from 15.0 to 87.2 mg/m<sup>3</sup>. Workers of the PVC division experienced exposure only to VC in the range from 1.1 to 10.7 mg/m<sup>3</sup>. The duration of the presence of workers of primary occupations in the workshops was 60-80% of the work shift, while workers of auxiliary occupations spent only 40-50% of their shift time in the workshops. Thus, workers in auxiliary occupations experience lower ranges of exposure to toxicants.

The study was carried out in two stages. During the first stage, urine samples were collected for biological monitoring from 65 workers at a medical examination carried out at a company clinic during the period of long rest. The mean age and work experience of participants were 45.1 (SD 9.6) and 18.7 (SD 6.7) years, respectively. The interval between the last exposure and the collection of urine samples ranged from 15 to 64 hours. At the second stage of the study, 10 workers working in three consecutive shifts were studied. The group consisted of workers of the primary professions with healthy lifestyles (not smoking and not drinking alcohol), regularly providing a urine sample at the end of the work shift, as well as on the following day before the beginning of their shift. Their mean age and work experience were 34.5 (SD 7.0) and 7.1 (SD 4.7) years, respectively. The results were evaluated in relation to a control group of 34 people with a mean age of 32.3 (SD 8.7) who did not work at this company and were thus not exposed

to the toxicants.

### Gas Chromatography of Urine Samples

Urine samples were collected in polypropylene containers and stored at  $-20\text{ }^{\circ}\text{C}$  until being analyzed. An investigation of the urinary TDAA content was carried out on an Agilent 7890A gas chromatograph with an Agilent 5975C mass-selective detector.<sup>11</sup> An 0.1-mL aliquot of the urine sample to be analyzed was added to 0.1 mL of a boron trifluoride (10%) solution in methanol and placed in a 1.5-mL glass chromatographic vial; it was then thermostated for 15 min at  $80\text{--}85\text{ }^{\circ}\text{C}$ . After cooling, 0.5 mL of ethylacetate and 0.9 mL of 180 mg/mL sodium sulfate solution was added. The samples were shaken for five minutes and centrifuged at 3000 rpm for three minutes. After layer separation, the upper organic layer was analyzed.

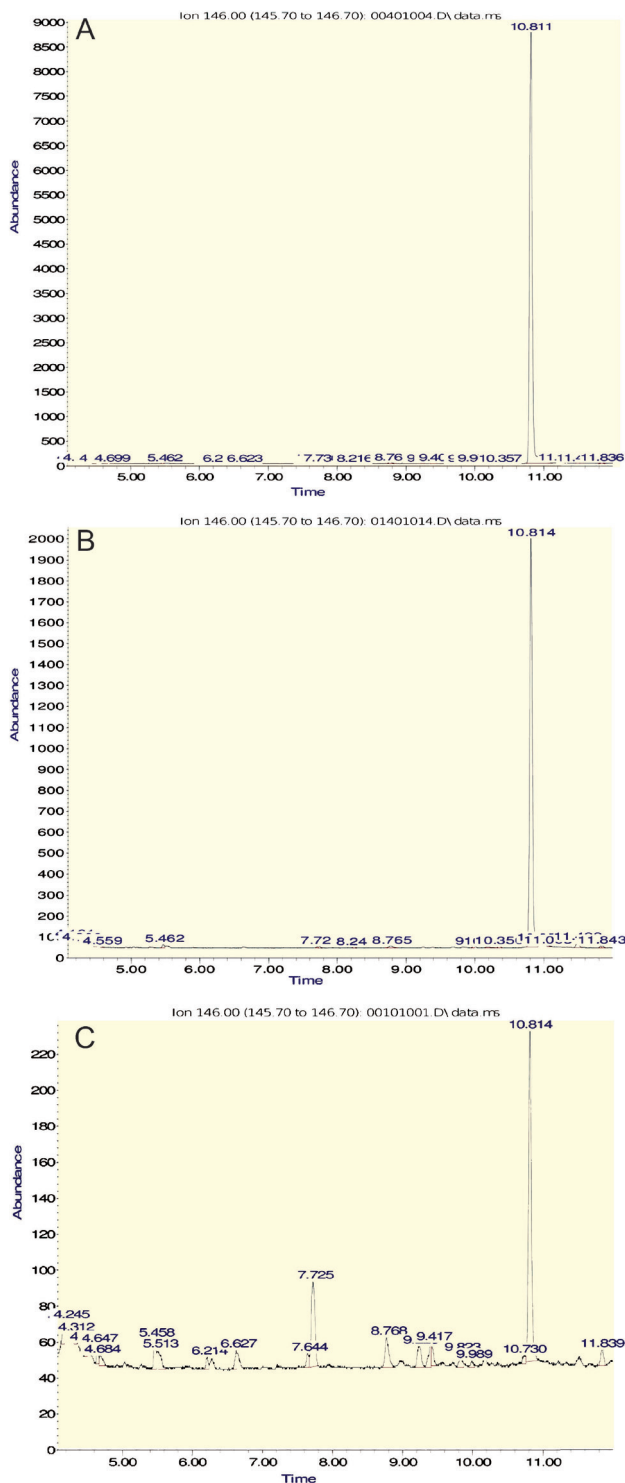
### Conditions of GC-MS analysis

We used HP-5MS column ( $30.0\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ ), a helium flow rate of 1.0 mL/min through the column, and an injector temperature of  $250\text{ }^{\circ}\text{C}$ . It was splitless for 0.3 min and had a purge flow rate to split vent of 40 mL/min. The oven column was  $80\text{ }^{\circ}\text{C}$  hold for 1 min at a rate of  $5\text{ }^{\circ}\text{C}/\text{min}$  to reach  $130\text{ }^{\circ}\text{C}$  hold for 1 min. The temperatures of interface was  $280\text{ }^{\circ}\text{C}$ ; ion source EI,  $230\text{ }^{\circ}\text{C}$ ; and quadrupole,  $150\text{ }^{\circ}\text{C}$ . The solvent delay was 4 min; means of mass-chromatogram registration was SIM (146, 178).

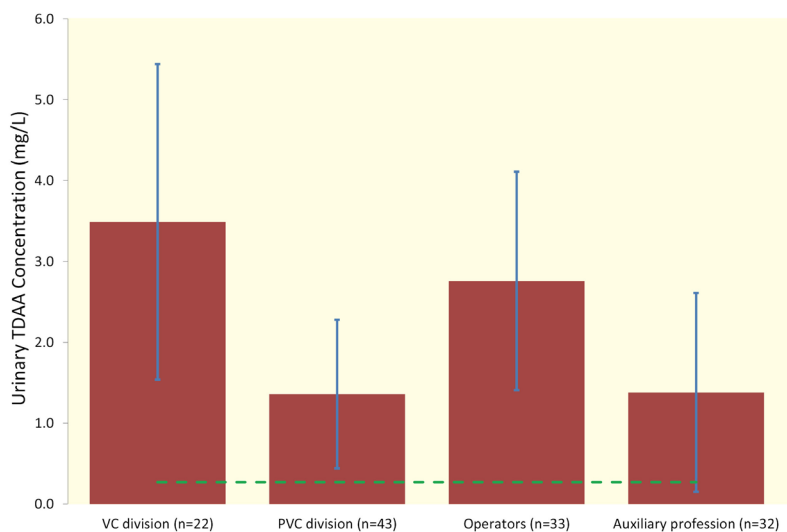
Based on known urinary concentrations of TDAA, the limit of detection (LOD) and limit of quantitation (LOQ) were estimated to be 0.02 and 0.06 with signal-to-noise ratios of 3/1 and 10/1, respectively. Examples of mass chromatograms are presented in Figure 1.

### Ethics

The protocol of this study was approved by the Ethical Committee of the East Si-



**Figure 1:** Mass chromatograms of urine sample: A) operator, B) auxiliary professions, and C) control group; RT = 10.8 min



**Figure 2:** Mean urinary TDAA concentrations in the studied VC and PVC production workers. The green dashed line represents the concentration in the control group. Error bars represent 95% CI of the means.

berian Institute of Medical and Ecological Research. The work did not infringe on the rights or endanger the well-being of the workers surveyed and was conducted in accordance with the Helsinki Declaration of the World Association on Ethical Principles of Scientific Medical Research with Human Participation, as amended in 2000.

### Statistical Analysis

Statistika ver 6.1 software was used for statistical analysis using Kruskal-Wallis and Mann-Whitney U tests with Bonferoni correction, one-sample Wilcoxon rank test, and *Student's t* test for paired data. The normality of the distribution of quantitative variables was examined with Shapiro-Wilk test.

### Results

The mean urinary concentration of TDAA in VC production division workers was 2.57-fold higher than that in PVC production division workers ( $p < 0.05$ , Fig 2). The mean concentrations of TDAA in the urine of workers in these divisions were 5.0 and 19.9 times higher than that in the control group (0.27, SD 0.13 mg/L). The proportion of urine samples with TDAA levels exceeding the metabolite level in the control group was higher in VC production workers (91%) compared with PVC production workers (74%).

The mean concentration of urinary TDAA in VC and PVC production operators was two times higher than those of the auxiliary occupations group ( $p < 0.05$ ). The percentage of urine samples with TDAA levels exceeding the levels in the control group was also higher among operators (85%) compared with the auxiliary workers (75%) (Table 1).

The mean TDAA level in VC production operators was 3.79 (SD 4.5, range 0.18 to 13.29) mg/L, 3.21 times higher than in that in PVC production operators (1.18, SD 1.01, range 0.12 to 2.71 mg/L) ( $p < 0.05$ ). The percentage of the urine samples with TDAA level exceeding the metabolite level in the control group was also higher in VC production operators (90%) compared with PVC production operators (77%).

Workers of the primary occupations of the PVC shop had similar changes in their

### TAKE-HOME MESSAGE

- Low concentrations of vinyl chloride monomer (VC) and 1,2-dichloroethane have mutagenic, carcinogenic, hepatotropic, psychotropic, and irritating effects on the body.
- These chemicals are metabolized by liver to thiodiacetic acid (TDAA) and 2-chloroethylene oxide, monochloroacetic acid.
- Excretion of TDAA into urine of workers in VC and PVC workshops was significantly higher than that in an unexposed control group. The urinary levels of the metabolites depend on the degree of exposure to organochlorine toxicants.

**Table 1:** Urinary concentrations of TDAA in VC and PVC production workers stratified by profession and time elapsed since the last exposure.

Time elapsed since the last exposure (hrs)	Mean (SD) [range] urinary TDAA concentrations, mg/L	Percent of urine samples with TDAA levels exceeding the control level, 0.27 (SD 0.13) mg/L
Operators		
16–17 (n=6)	0.96 (0.71) [0.56 to 2.39]	100
24 (n=17)	2.44 (3.8) [0.18 to 13.29]	82
48 (n=10)	4.38 (4.4) [0.12 to 11.83]	80
Auxiliary professions		
15–17 (n=14)	0.8 (0.73) [0.18 to 2.76]	71
24 (n=15)	2.17 (4.9) [0.26 to 19.12]	93
41–64 (n=3)	0.15 (0.1) [0.07 to 0.26]	0

urinary TDAA level before and after the shift compared to the post-exposure period (Table 2). The urinary levels of TDAA operators and cleaners were 2.1–3.0-fold higher than those at the end of the previous day shift and before the beginning of the next day shift (12 hours after the end of the shift,  $p < 0.05$ ). Concentrations of TDAA in the urine of workers before the first day shift after a 3-day weekend off and before the night shift after a 2-day inter-shift rest showed no variance. However, after the end of the night shift, the levels of TDAA in the urine of workers were 2.6 times higher than that measured before the beginning of this shift. The highest percentage of urine samples with TDAA concentrations exceeding the levels of the control group was noted before and after the second day shift (Table 2).

## Discussion

Our study showed that excretion of TDAA into urine of workers in VC and PVC workshops was significantly higher than that in an unexposed control group and that it depended on the degree of exposure to organochlorine toxicants. In VC production

division workers and in the those working in primary professions (operators) who experience higher levels of exposure, the concentration of TDAA in the urine was significantly higher than that of those working in PVC workshop and auxiliary professions ( $p < 0.05$ ). Previous studies have shown that among workers exposed to VC at concentrations  $> 5 \text{ mg/m}^3$ , the TDAA excretion is dose-dependent.<sup>10</sup> We found that in the urine collected at the beginning of the next shift, the level of TDAA was significantly higher in operators, those with higher exposure, than in those with moderate and low exposure (*ie*, technicians and workers of treatment facilities).

It should be noted that the excretion dynamics of TDAA into urine has specific characteristics and does not always directly depend on the level of exposure to VC. Regarding the metabolism of VC, several animal models show that the intake of VC ceases as soon as there is an equilibrium between the concentration in the blood and the inhaled VC concentration.<sup>7,8,12</sup> If nearly 100% of the substance enters the body orally, then the initial VC that is not metabolized on the first passage through the liver is excreted. The amount of ex-



**Table 2:** Urinary TDAA concentrations in PVC producing workers before and after shift work. Values are mean (SD), [range], and percentage of urine samples with TDAA concentrations exceeding the control group level, 0.27 (SD 0.13) mg/L.

Workers	First day shift		Work shift following the first day shift		Night shift	
	Before (after 3 days off)	After (>12 hrs of work)	Before (after 12 hrs of rest)	After (>12 hrs of work)	Before (after 2 days off)	After (>12 hrs of work)
All workers (n=10)	0.41 (0.2) [0.20 to 0.77] 60%	0.52 (0.41) [0.12 to 1.32] 60%	1.21 (0.53) [0.55 to 2.46] 100%	0.78 (0.52) [0.3 to 1.8] 100%	0.39 (0.25) [0.1 to 0.86] 60%	1.01 (0.39) [0.27 to 1.68] 90%
Operators (n=6)	0.30 (0.1) [0.20 to 0.42] 33%	0.67 (0.49) [0.12 to 1.32] 67%	1.40 (0.62) [0.55 to 2.46] 100%	0.9 (0.64) [0.35 to 1.8] 100%	0.27 (0.08) [0.15 to 0.34] 50%	1.08 (0.49) [0.27 to 1.68] 83%
Cleaners (n=4)	0.59 (0.2) [0.31 to 0.77] 100%	0.30 (0.07) [0.23 to 0.4] 50%	0.94 (0.19) [0.77 to 1.15] 100%	0.61 (0.23) [0.3 to 0.82] 100%	0.57 (0.33) [0.1 to 0.86] 75%	0.90 (0.14) [0.71 to 1.01] 100%

haled VC increases with increasing exposure level and the relative excretion into the urine of metabolites decreases, indicating a saturation of the metabolism.<sup>13</sup>

Cheng, *et al*,<sup>10</sup> suggested that the level of TDAA in urine is best detected between the end of one shift and the beginning of the next shift, and that the level can be used to assess the risk of exposure to workers in PVC production. Our findings were consistent with these results and clearly showed that the urinary concentrations of TDAA in workers exposed to VC and EDC during their shift reached a maximum after 12 hours of rest following the end of the work shift and at the beginning of the next shift. In the meantime, the dynamics of TDAA excretion into the urine of workers of PVC department before and after the shift work had the same pattern of changes in the level of metabolite during successive shift works. The observed relatively low excretion of TDAA into the urine of workers at the end of the shift (especially, among cleaners) is probably due in part to the phenomenon of saturation of the metabolism of VC and EDC caused by the destruction of the cytochrome P-450

protein due to the intake of a large number of hydrocarbons, and its consequent nonlinear kinetics of the metabolic pathway for these toxicants.<sup>8,14</sup> The higher urinary concentrations of TDAA in workers after the end of the night shift compared with the beginning of the shift, is possibly related to a relatively low exposure to the toxicants, which does not saturate the metabolic pathway, due to the stopping of PVC production for preventive maintenance according to technological regulations. Therefore, we confirmed the previously stated assumption and concluded that the optimal time for collecting urine samples from workers in PVC production for bio-monitoring of exposure to VC and EDC during operation is at the beginning of the next shift, 12 hours following the cessation of exposure to toxicants.

Exploring the dynamics of the excretion of TDAA in the urine of workers during a long rest, we found that the highest urinary concentrations of TDAA were recorded in the primary occupations (operators), 24–48 hours, and in auxiliary professions 24 hours after the termination of the exposure to organochlorine toxicants.

We assume that the revealed differences in the dynamics of excretion of TDAA in the urine of workers are associated with the above-mentioned features of the biotransformation of these toxicants and the varying exposure among primary and auxiliary professions workers. It was possible to see that the metabolite excretion in workers in auxiliary professions exposed to the least impact of the toxicants occurred much faster than in those working in the primary professions, reaching the level recorded in the control group. In animal studies, it was noted that the more VC enters the body, the longer its metabolites are in the tissues.<sup>15</sup> Therefore, the established temporal characteristics can be used in biomonitoring studies during a long rest for workers after exposure to VC and PVC.

In conclusion, in biomonitoring studies of the urinary concentration of TDAA, a biomarker of organochlorine compounds, in workers producing PVC, it is recommended to collect urine in the process of work, 12 hours after the work shift, before the next shift, or during a long rest, 24–48 hours following the cessation of exposure to the toxicants.

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**Conflicts of Interest:** None declared.

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