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Int. Arch. Occup. Environ. Hlth. 41, 199–205 (1978)

International Archives of Occupational and Environmental Health

Determination of Thiodiglycolic Acid in Urine Specimens of Vinyl Chloride Exposed Workers

G. Müller[1], K. Norpoth[1], E. Kusters[2], K. Herweg[3] and E. Versin[2]

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Summary. A strong correlation was found between vinyl chloride concentrations at working places and the increased excretion of thiodiglycolic acid of 18 exposed workers. The mean air concentration of vinyl chloride was calculated referring to personal exposure. The values obtained were in the range of 0.14 – 7,0 ppm. The excretion of thiodiglycolic acid – measured by GC-MS analysis – amounted to 0,3 – 4.0 mg/L. It could be demonstrated that significant increases of the metabolite excretion occur even at VC-concentrations below 5 ppm.

Key words: Vinyl chloride – Thiodiglycolic acid – vinyl chloride metabolism in man – Vinyl chloride urine metabolites – Biological exposure control

Today the air concentrations of vinyl chloride at working places are rigorously reduced mainly due to its carcinogenic [5, 14, 16, 19, 20–23, 34] and mutagenic [6, 8, 9, 14, 15, 18, 19, 30] properties. Exposure controls are commonly based on air analyses by means of highly sensitive, but not always strongly specific analytical methods.

Therefore, we emphasized the usefulness of additional biological exposure controls [24, 26].

By animal experiments we could demonstrate the possibility of determining the excretion of thiodiglycolic acid and S-carboxy-methylcysteine, two urine metabolites of vinyl chloride [10, 24, 26, 27, 35]. Data of thiodiglycolic acid in the urine of workers slightly exposed to vinyl chloride and the improved analytical method used for determination are reported here.

Materials and Methods

Chemicals

Thiodiglycolic acid (98% purity) was purchased from Janssen Pharmaceutica, Dept. Aldrich Europe, B-2340 Beerse, Belgium. Diazomethane was generated from N-methyl-N-nitroso-p-toluolsulfonamide according to de Boer and Backer [3]. All other substances were purchased at analytical grade from E. Merck, A.G., D-6100 Darmstadt.

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Procedures

Determinations of Individual Exposure by Personal Air Sampling

Sampling

Sampling Tube. Traces of organic pollutants among which vinyl chloride in the factory air were collected by glass tube samplers with a length of 12 cm and an internal diameter of 3 mm, filled with 25–50 mesh charcoal [7]. These charcoal tubes had a breakthrough volume for vinyl chloride at 20° C of about 5 liters [33].

Pump. To draw the air through the tube samplers we used a small pump-carried personal sampler manufactured by Anatone J. Sipin Co. The sampled air volume can be calculated from the number of pistonplays of the pump, registered by a built in counter.

As the amount of charcoal and the density of packing varied from sampling tube to sampling tube, we had to calibrate the pump before each experiment. The relationship between pistonplays and normal gas volume was easily measured with a gasbubble meter and a stopwatch.

Analysis

Analytical Conditions. A special injection port that allows for direct introduction of the total sample, cartridge included, was used for the thermal desorption of the trapped vinyl chloride [1, 29]. The vaporized vinyl chloride was routed to a gas chromatographic column. To separate the vinyl chloride from other sampled organic vapor pollutants we used a 25 m wall coated carbowax 20 M capillary at 80° C. The detector used was, of course, a flame ionization detector. The surface of the vinyl chloride peak was measured by an integrating computer.

Calibration. A calibration gas was dynamically prepared by passing a known gasstream over a teflon permeation tube filled with liquid vinyl chloride [28, 31]. The concentration of vinyl chloride was calculated by the loss of weight of the permeation tube over several days. A known volume of this gas was drawn through a charcoal tube and this tube was analyzed as described above. The gaschromatographic results were reported as the amount of μg absorbed by the charcoal tube sampler.

Evaluation of the Personal Exposure Data. The mean concentration of vinyl chloride in the air during the experiment was calculated from the amount of absorbed vinyl chloride analyzed by gaschromatography and from the sampled air volume calculated from the number of pistonplays of the pump during sampling.

VC-Determination in the Air of Working Places. The measurements were performed using multipoint flame ionization detectors (FID, type RAID 1511, spread in the working area. Total hydrocarbons in the air of each sampling point were analyzed once a minute. It has been shown by GC-measurements that approximately 2 ppm of the total hydrocarbons in this working area are not VCM. Considering this amount of 2 ppm not – VCM – hydrocarbons the true air concentration of vinyl chloride monomer was calculated. The personal exposure of each worker was evaluated. Thus from registrations based on the duration of stay at different working places, all values of VCM air concentrations given in this paper, which are not obtained by personal air sampling, refer also to personal exposure.

Determination of Thiodiglycolic Acid by GC-MS-Analysis. In a 10 ml glass tube 1 ml of urine and 0,3 ml of conc. HCl were evaporated to dryness in vacuo at room temperature. The residue was suspended in methanol and the suspension was completely methylated by means of diazomethane in ethyl ether [3]. After 15 minutes Stock ion exchange resin (Dowex 50 WX 8, H⁺) were added. Thiodiglycolic acid dimethyl ester was determined by gaschromatographic separation and single ion detection of the mass fragment M 146. It is necessary to use the standard addition technique to obtain exact measurements.

Fig. 1. Urine concentrations of thiodiglycolic acid (TDGA). Group A: 20 non-exposed males. Group B: workers exposed to 0.14 – 3.35 ppm VCM measured by FID air analysis. Group C: 8 workers exposed to 1.02 – 7.0 ppm VCM measured by use of personal air samplers

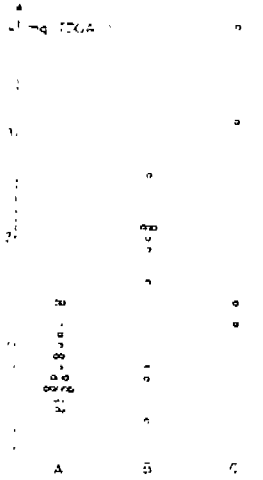

Fig. 2. Urine concentrations of thiodiglycolic acid (TDGA) after classification of 87 data according to four collectives which are related to four different VCM exposure ranges. A = 0.6 ppm, B = 0.01 – 2.50 ppm, C = 2.51 – 5.00 ppm, D = 5.01 – 7.50 ppm

Conditions

Gaschromatograph 9500 combined with mass spectrometer 3200 (Finnigan). Column 1.5 m × 0.3 cm 3 % OV 1 on 60/80 DMCS Chromosorb W. Carrier gas 30 ml/min. Column temperature 130° C. Injection port temperature 200° C. Separator temperature 200° C.

Results

The analytical procedure which we developed for sufficiently precise determination of small amounts of thiodiglycolic acid in urine samples allowed us to compare data of two groups of VC-workers with those of a non-exposed group.

As demonstrated by Fig. 1, it is possible to distinguish between normal values (group A) and such obtained from slightly exposed workers (group B and C) at the 1 % significance level. As we calculated from our normal values, in all cases concen-

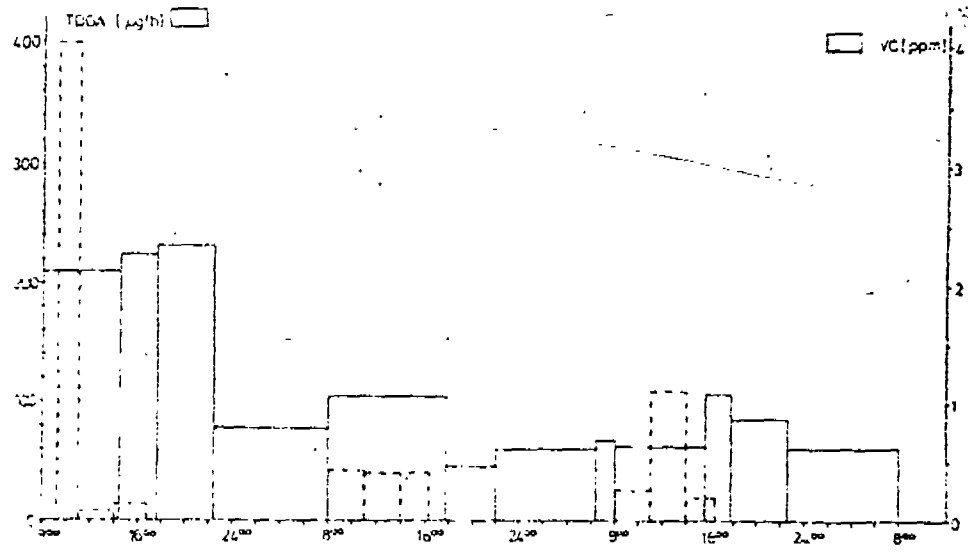

Fig. 3. Results of a comparison investigation on VCM exposure levels and the excretion of thiodiglycolic acid (TDGA)/hour in the case of a single worker, who was observed during a threeday period

trations of thiodiglycolic acid exceeding 2.1 µg/ml urine can be distinguished from normal concentrations at the 1 % significance level (99 % probability of confidence). Those values were found twice in group B and five times in group C. The mean values of the three groups suggest a correlation between the extent of exposure and the amount of thiodiglycolic acid excreted. This correlation could be proved by classification of the analytical data according to four classes of different exposure. As shown in Fig. 2, an increase of metabolite concentrations in the urine is found with increasing exposure. The correlation was tested by a distribution free statistical procedure [17] and could be confirmed at the level of $p < 0{,}01$.

Since individual exposure control by means of personal air sampling seems to provide more precise data than global air analysis, we started exact examinations of single workers controlling the personal exposure during time periods of two or three days. In this period all urine portions were collected separately and in each of them the concentration of thiodiglycolic acid was measured. The results of one investigation are shown in Fig. 3. It can be seen that the increase of metabolite excretion began shortly after starting the exposure each day and that the highest concentrations were found in the first or second urine sample obtained after the highest possible VC-uptake. Furthermore, it was a result of this investigation that the metabolite excretion exceeded the normal range significantly even after a mean exposure level of about 1,5 ppm but not after an individual exposure of less than 1,0 ppm.

Discussion

Main metabolites of vinyl chloride which are excreted in the urine of laboratory animals are thiodiglycolic acid [16, 24, 26, 27, 35], S-carboxy-methylcysteine [11, 24, 26, 27] and hydroxyethyl mercapturic acid [11, 35].

Previously, we proposed quantitative determinations of thiodiglycolic acid and S-carboxy-methyl cysteine in the urine of vinyl chloride exposed workers for biological exposure control [24–26]. In the meantime, we could demonstrate by animal experiments that S-carboxy-methyl cysteine, a precursor of the final metabolite thiodiglycolic acid, accumulates only in the case of vinyl chloride air concentrations higher than 500 ppm in the exposure chamber [25], which are unusual today at working places. Concerning hydroxyethyl mercapturic acid measurements where performed using an amino acid analyzer. No amounts where found in the urine of 18 workers slightly exposed to vinyl chloride which exceeded the corresponding values of thiodiglycolic acid. It may be taken into consideration that the contribution of alternative metabolic routes to the biotransformation of vinyl chloride depends on the exposure level [13, 25, 36]. Thus it is not yet clear, whether hydroxyethyl mercapturic acid is the main urine metabolite of VCM in man or whether it is thiodiglycolic acid. Investigating the real concentration of the latter metabolite, a more precise procedure was needed than in our animal experiments performed previously [18, 20] because its increase after VCM exposure is apparently less marked in man than in the rat. Furthermore, it has been shown that thiodiglycolic acid is also a naturally occurring urine metabolite in man [25]. Using an improved analytical method then, we could clearly demonstrate that the metabolite concentrations in the urine of workers exceed the normal level significantly even after VC-exposures between 1.5 and 5 ppm. This increase of urine concentrations depends clearly on the exposure level.

Neglecting other elimination routes than urinary excretion theoretical values of the daily amounts (E) of excreted thiodiglycolic acid may be calculated according to the following concerning:

$$E = \frac{V_{8h} \times C_{VC} \times R_{VC} \times Q \times MG_{TDGA}}{MG_{VC}}$$

The explanation of the symbols is:

V_{8h} = lung ventilation during an 8 hour intervall

C_{VC} = mean air concentration of VC

R_{VC} = resorption value, which has been found in animals as about 0,4 (4)

Q = relative amount of thiodiglycolic acid as compared to the total amount of VC-metabolites, which has been found between 25 % (35) and 50 % (13).

MG_{TDAA} = molecular weight of thiodiglycolic acid

MG_{VCM} = molecular weight of VCM.

The thus determined theoretical values correspond well to the measured excretion rates in the rat. They are, however, between 2 and 10 times higher than the excretion values as shown in Figs. 1 and 2. This may be due to different biotransformation rates in rat and man. On the other hand, the values obtained by personal air sampling are more in accordance with the theoretical values than those obtained by direct air analyses using FID-detectors. In the latter case, as mentioned previously, other hydrocarbons are measured together with VCM amounting to an average level of

about 2,0 ppm. This average may have been exceeded during some of our measurements. Thus, more evidence is needed for the true slope of the function between VCM exposure and excretion of thiodiglycolic acid. Therefore, we started a new study to reinvestigate this slope by measuring the personal VCM exposure using the personal air sampling technique in direct comparison with the use of FID-detectors for air analyses.

As shown in Fig. 3, even a slight VCM exposure can cause characteristic fluctuations of thiodiglycolic acid excretion and this can be revealed by continuous urine analysis. Considering the excreted amounts of the VCM metabolite per hour, it seems possible to prove a periodical uptake of very low amounts of VCM by single workers. Such measurements could also provide some informations about inter- and intraindividual differences of VCM metabolism in man.

Acknowledgement. This study was supported by a grant of the Berufsgenossenschaft der Chemischen Industrie, D-6900 Heidelberg 1, BRD.

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Summary. A strong correlation was found between vinyl chloride concentrations at working places and the increased excretion of thiodiglycolic acid of 18 exposed workers. The mean air concentration of vinyl chloride was calculated referring to personal exposure. The values obtained were in the range of 0.14 – 7.0 ppm. The excretion of thiodiglycolic acid – measured by GC-MS analysis – amounted to 0,3 – 4.0 mg/L. It could be demonstrated that significant increases of the metabolite excretion occur even at VC-concentrations below 5 ppm.

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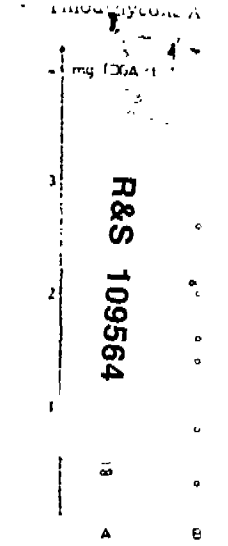

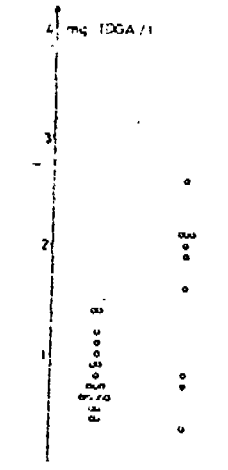

Conditions

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Results

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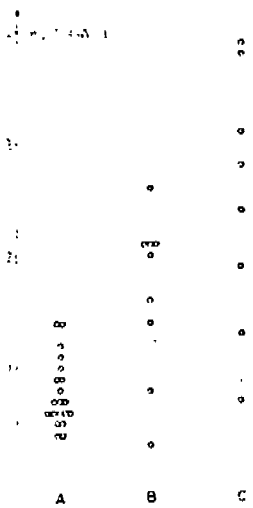

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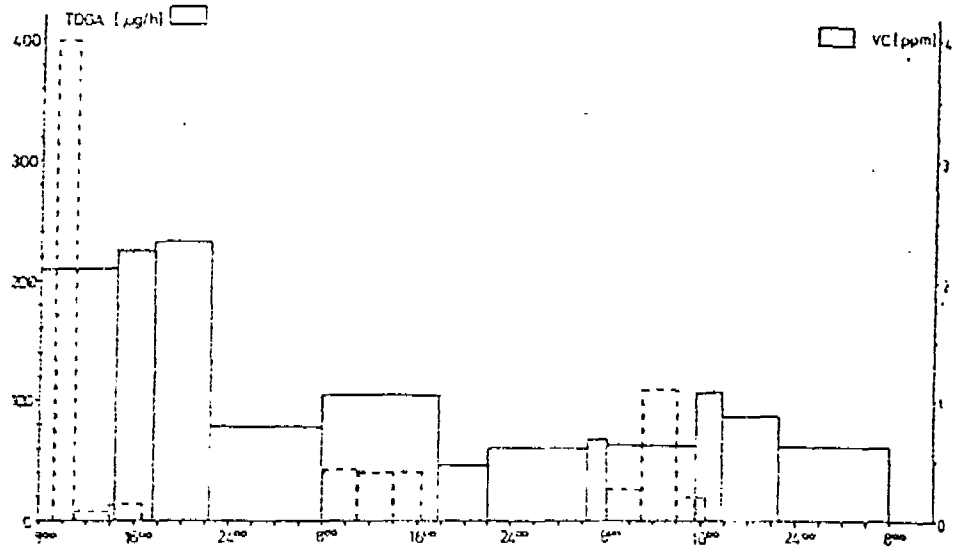

Fig. 3. Results of a comparison investigation on VCM exposure levels and the excretion of thiodiglycolic acid (TDGA)/hour in the case of a single worker, who was observed during a three-day period.

trations of thiodiglycolic acid exceeding 2,1 µg/ml urine can be distinguished from normal concentrations at the 1 % significance level (99 % probability of confidence). Those values were found twice in group B and five times in group C. The mean values of the three groups suggest a correlation between the extent of exposure and the amount of thiodiglycolic acid excreted. This correlation could be proved by classification of the analytical data according to four classes of different exposure. As shown in Fig. 2, an increase of metabolite concentrations in the urine is found with increasing exposure. The correlation was tested by a distribution free statistical procedure [17] and could be confirmed at the level of $p < 0{,}01$.

Since individual exposure control by means of personal air sampling seems to provide more precise data than global air analysis, we started exact examinations of single workers controlling the personal exposure during time periods of two or three days. In this period all urine portions were collected separately and in each of them the concentration of thiodiglycolic acid was measured. The results of one investigation are shown in Fig. 3. It can be seen that the increase of metabolite excretion began shortly after starting the exposure each day and that the highest concentrations were found in the first or second urine sample obtained after the highest possible VC-uptake. Furthermore, it was a result of this investigation that the metabolite excretion exceeded the normal range significantly even after a mean exposure level of about 1,5 ppm but not after an individual exposure of less than 1.0 ppm.

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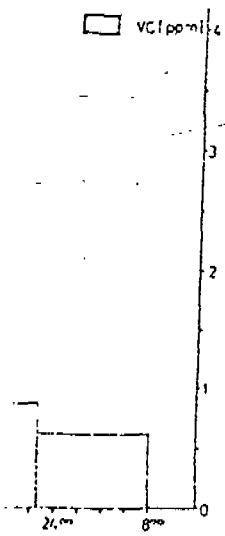

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Discussion

Main metabolites of vinyl chloride which are excreted in the urine of laboratory animals are thiodiglycolic acid [10, 24, 26, 27, 35], S-carboxy-methylcysteine [11, 24, 26, 27] and hydroxyethyl mercapturic acid [11, 35].

Previously, we proposed quantitative determinations of thiodiglycolic acid and S-carboxy-methyl cysteine in the urine of vinyl chloride exposed workers for biological exposure control [24, 26]. In the meantime, we could demonstrate by animal experiments that S-carboxy-methyl cysteine, a precursor of the final metabolite thiodiglycolic acid, accumulates only in the case of vinyl chloride air concentrations higher than 500 ppm in the exposure chamber [25], which are unusual today at working places. Concerning hydroxyethyl mercapturic acid measurements where performed using an amino acid analyzer. No amounts where found in the urine of 18 workers slightly exposed to vinyl chloride which exceeded the corresponding values of thiodiglycolic acid. It may be taken into consideration that the contribution of alternative metabolic routes to the biotransformation of vinyl chloride depends on the exposure level [13, 25, 36]. Thus it is not yet clear, whether hydroxyethyl mercapturic acid is the main urine metabolite of VCM in man or whether it is thiodiglycolic acid. Investigating the real concentration of the latter metabolite, a more precise procedure was needed than in our animal experiments performed previously [18, 20] because its increase after VCM exposure is apparently less marked in man than in the rat. Furthermore, it has been shown that thiodiglycolic acid is also a naturally occurring urine metabolite in man [25]. Using an improved analytical method then, we could clearly demonstrate that the metabolite concentrations in the urine of workers exceed the normal level significantly even after VC-exposures between 1.5 and 5 ppm. This increase of urine concentrations depends clearly on the exposure level.

Neglecting other elimination routes than urinary excretion theoretical values of the daily amounts (E) of excreted thiodiglycolic acid may be calculated according to the following concerning:

$$E = \frac{V_{8h} \times C_{VC} \times R_{VC} \times Q \times MG_{TDGA}}{MG_{VC}}$$

The explanation of the symbols is:

V_{8h} = lung ventilation during an 8 hour intervall

C_{VC} = mean air concentration of VC

R_{VC} = resorption value, which has been found in animals as about 0,4 (4)

Q = relative amount of thiodiglycolic acid as compared to the total amount of VC-metabolites, which has been found between 25 % (35) and 50 % (10).

MG_{TDAA} = molecular weight of thiodiglycolic acid

MG_{VCM} = molecular weight of VCM.

The thus determined theoretical values correspond well to the measured excretion rates in the rat. They are, however, between 2 and 10 times higher than the excretion values as shown in Figs. 1 and 2. This may be due to different biotransformation rates in rat and man. On the other hand, the values obtained by personal air sampling are more in accordance with the theoretical values than those obtained by direct air analyses using FID-detectors. In the latter case, as mentioned previously, other hydrocarbons are measured together with VCM amounting to an average level of

about 2,0 ppm. This average may have been exceeded during some of our measurements. Thus, more evidence is needed for the true slope of the function between VCM exposure and excretion of thiodiglycolic acid. Therefore, we started a new study to reinvestigate this slope by measuring the personal VCM exposure using the personal air sampling technique in direct comparison with the use of FID-detectors for air analyses.

As shown in Fig. 3, even a slight VCM exposure can cause characteristic fluctuations of thiodiglycolic acid excretion and this can be revealed by continuous urine analysis. Considering the excreted amounts of the VCM metabolite per hour, it seems possible to prove a periodical uptake of very low amounts of VCM by single workers. Such measurements could also provide some informations about inter- and intraindividual differences of VCM metabolism in man.

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