Relation between the iodine azide test and the TTCA test for exposure to carbon disulphide

J ROSIER,¹ G BILLEMONT,² C VAN PETEGHEM,¹ M VANHOORNE,² R GROSJEAN,³ AND A VAN DE WALLE³

From the Laboratorium voor Bromatologie,¹ Farmaceutisch Instituut, Rijksuniversiteit van Gent, 9000 Gent, Dienst voor Hygiene en Sociale Geneeskunde,² Academisch Ziekenhuis, Rijksuniversiteit van Gent, 9000 Gent, and Laboratorium voor Industriële Toxicologie,³ Ministerie van Arbeid en Tewerkstelling, B-1040 Brussel, Belgium

ABSTRACT Exposure to carbon disulphide (CS₂) in a viscose plant was measured by personal monitoring and by application of the iodine azide test and quantification of 2-thio-thiazolidine-4-carboxylic-acid (TTCA) in urine samples. A relation was found between the rise in urinary TTCA concentration during the workshift and the exposure index E₁. The correlation (r) between the exposure index and the atmospheric concentrations of CS₂ in workroom air below 100 mg CS₂/m³ was 0.59 (n = 9). The correlation between the increase in TTCA concentrations during the workshift and the atmospheric CS₂ concentrations was found to be higher when urine samples at the end of the workshift with creatinine concentrations below 1 mg/ml and above 3 mg/ml were disregarded (from r = 0.61; n = 20 to r = 0.84; n = 14). A high correlation was found (r = 0.86; n = 13) when the end of workshift urine samples were analysed, provided that their creatinine concentrations are not beyond the limits given above.

The iodine azide test which is based on the fact that certain constituents in the urine of rabbits exposed to carbon disulphide catalyse the reaction between iodine and sodium azide, was developed by Vasak *et al*¹ to evaluate the degree to which workers are exposed to carbon disulphide. The chemical reaction may be written as:

The original method consisted of measuring the time needed for the disappearance of the yellowish brown colour of iodine on the addition of urine to the reaction medium. Vasak proposed an exposure index E in which

$$E = C \log t$$

where E = exposure index,

C = creatinine concentration in mg per ml urine, and

t = discoloration time in seconds.

The reaction between sodium azide and iodine proceeds slowly at room temperature but is apparently catalysed by the addition of a number of organic and inorganic compounds. This is thought to be due to the formation of an intermediary addition compound of the catalysing agent with iodine or sodium azide, which will then react with sodium azide or iodine respectively, forming nitrogen and iodide ions, and leaving the catalysing agents unchanged.² The catalysing agents responsible for this reaction are most likely to be dithiocarbamate derivatives of amino acids and some other CS₂ metabolites such as 2-mercaptothiazolinone-5³ and thiourea,⁴ which when added in pure form to the iodine azide solution, discolours it immediately. Some sulphur containing compounds derived from drugs or the diet, however, also catalyse the reaction, thus giving false positive results. In addition, the main disadvantage of the method is that discoloration occurs gradually, and there is no specific point of discoloration. Because of the subjective element in the readings, different exposure indices could be determined when the urine sample is added to the reaction mixture.

In 1981 Van Doorn discovered 2-thio-thiazolidine-4-carboxylic-acid (TTCA) in the urine

Received 25 April 1983
Accepted 11 July 1983

of workers occupationally exposed to CS₂.⁵ TTCA is a heterocyclic five membered ring that is formed in the body by the reaction between carbon disulphide and glutathione.⁵ The quantification of TTCA in the urine of viscose workers thus seems to have interesting possibilities for the biological monitoring of CS₂ exposure.⁶

Materials and methods

PERSONAL MONITORING

The concentration of CS₂ in the workroom air was estimated over eight hours by means of personal monitoring pumps on workers from the different departments of a viscose plant (see Rosier *et al*⁶ for further details.) From each group of shift and day workers (7 to 8 workers per group) two were asked to wear a personal monitoring pump, with the charcoal tube attached as closely as possible to the head in order to estimate the CS₂ concentration in the breathing zone. The charcoal tubes were replaced after two hours, and sampling was carried out at periods of 120 to 360 minutes throughout the eight hour working period. CS₂ concentrations were measured according to the NIOSH method SV 248⁷ as described elsewhere.⁶

Biological monitoring

URINE SAMPLES

The urine samples were collected at the beginning and at the end of the workshift and immediately deep frozen and stored at -22°C until analysis.

Urine samples from the shift workers were taken during the last two days of a working period and at the beginning of a new period. In this way the TTCA concentrations obtained were directly related to the preceding exposure. After thawing, sediments were centrifuged off and the supernatant used for analysis.

MODIFIED IODINE AZIDE TEST

The test solution was prepared as prescribed by Djuric *et al*.⁸ Discoloration in the solution was monitored using a photometer (Eppendorf) equipped with a 366 nm filter and carried out at a temperature of 23°C; the discoloration time was measured as shown in fig 1. The test was considered negative when discoloration did not occur within three hours and the exposure index was calculated as mentioned above.

2-THIO-THIAZOLIDINE-4-CARBOXYLIC-ACID IN URINE

Urinary 2-thio-thiazolidine-4-carboxylic-acid was determined according to the method of Van Doorn.⁹

Fig 1 Discoloration curve of iodine azide reagent after adding urine of a worker exposed to carbon disulphide.

A high pressure liquid chromatograph (Spectra-Physics SP 8000) was used, equipped with a Hibar column (25.0 cm long and 4.6 mm ID, packed with Lichrosorb RP-18/5) and a variable wavelength UV detector (Spectra Physics SP 8400) set at 273 nm.

CREATININE ASSAY

The creatinine content of the urine samples was measured and analysed using a method based on the reaction of creatinine with alkaline picrate.

SYNTHESIS OF

2-THIO-THIAZOLIDINE-4-CARBOXYLIC-ACID

TTCA was synthesised according to the method of De Baun *et al*¹⁰ and purified by means of preparative high pressure liquid chromatography as described elsewhere.¹¹

SYNTHESIS OF AMMONIUMGLYCINE

DITHIOCARBAMATE

Ammoniumglycine dithiocarbamate was synthesised according to Jakubowski and Gessner as described elsewhere.¹²

Results

Table 1 shows the results of the iodine azide test and the concentrations of TTCA in the urine samples of the different workers together with their time weighted average (TWA) exposure values (in mg CS₂ per m³ of air) obtained by personal monitoring. As can be seen, the exposure to CS₂ is high; only four workers have TWA exposure values below the TLV value for CS₂ of 10 ppm.^{12,13} A description of their working conditions is given elsewhere.⁶

Figure 2 illustrates the relationship between the measured exposure indices (E) from end of work-shift urine samples and the corresponding TWA exposure values (TWA exposure = 168 - 16.7E; r

Table 1 Carbon disulphide exposure. Iodine azide test and TTCA test on urine samples

No	TWA	C	t	logt	E	TTCA, b	TTCa, e	TTCA, e(a)	TTCA, i
1	69.6	2.32	1659	3.22	7.47	1.8	3.71	7.62	1.91
2	160.2	2.32	24	1.37	3.19	1.67	12.39	25.44	10.27
3	96.6	2.04	129	2.11	4.31	2.97	15.79	28.51	12.82
4	134.6	0.96	c	c	c	2.94	4.14	3.52	1.2
5	136.9	2.40	20	1.31	3.15	0	8.12	17.25	8.12
6	112.9	2.36	19.0	1.28	3.02	0.53	16.21	33.85	15.68
7	80.3	3.96	c	c	c	2.52	13.6	47.66	11.08
8	146.0	2.44	5.02	0.70	1.71	1.33	17.61	38.02	16.28
9	89.4	1.84	422	2.62	4.83	1.55	10.78	17.55	9.23
10	117.2	0.96	c	c	c	2.13	10.98	9.33	8.85
11	144.2	0.92	c	c	c	2.37	7.37	6.00	5.00
12	160.5	0.52	c	c	c	2.28	9.94	4.57	7.66
13	28.4	1.52	f	f	f	0.50	3.38	4.55	2.88
14	59.8	1.24	10973	4.04	5.01	1.46	5.35	5.87	3.89
15	41.2	1.40	4047	3.61	5.05	0.72	2.32	2.87	1.60
16	25.1	2.44	3475	3.54	8.64	0.10	2.14	4.62	2.04
17	39.3	2.56	7499	3.87	9.92	0.10	2.37	5.37	2.27
18	8.7	2.96	415	2.62	7.75	d	d	d	d
19	31.5	1.52	7058	3.85	5.85	0.46	3.55	4.77	3.09
20	79.3	0.38	c	c	c	0.32	4.88	1.64	4.56
21	15.2	2.12	f	f	f	0.12	0.22	0.41	0.10

TWA = Time weighted average exposure value in mg CS₂/m³ of air.

C = Creatinine concentration in mg per ml urine.

t = Discoloration time in seconds.

E = Exposure index as defined by Vasak *et al.*¹

TTCA, b = TTCA concentration in mmol TTCA per mol creatinine; beginning of workshift urine samples.

TTCA, e = TTCA concentration (same units) in end of workshift urine samples.

(a) = TTCA concentration in end of workshift urine samples in 10⁻³ mmol TTCA per ml urine.

TTCA, i = Increase in TTCA concentration in mmol TTCA per mol creatinine.

c = Beyond creatinine limits set by Djuric *et al.*²

d = No beginning of workshift urine sample available.

f = Discoloration time greater than three hours.

= -0.82, n = 14, p = 0.05). The exposure indices of the entire group of beginning of workshift urine samples were negative. There is no correlation between the exposure indices E of the end of workshift urine samples and TWA exposure values (r = 0.17, p = 0.05) below 50 mg CS₂ per m³ of air, but table 2 shows that the correlation increases in proportion to the degree to which the results of increasing levels of TWA exposure values are taken into account.

Fig 2 TWA exposure values (in mg CS₂/m³ of air) related to exposure index E of end of workshift urine samples.

As may be seen in table 2, even if the results at TWA exposure values lower than 100 mg CS₂/m³ of air are taken into account, there is no clear correlation between E values and TWA exposure values.

There is a clear correlation, however, between the urinary TTCA increments and the exposure index (r = -0.79, n = 12, p = 0.05). When TTCA results are related to the TWA exposure values a lower correlation is found than when the E values are related to the TWA exposure values (fig 2) (r =

Table 2 Correlation coefficients (r) for the exposure index E and TWA exposure values at increasing levels of TWA exposure values

TWA exposure lower than:	r	n
41.2	0.18*	5
59.8	0.46*	6
69.6	0.29*	7
89.4	0.48*	8
96.6	0.60*	9
112.9	0.72†	10
136.9	0.77†	11
146.6	0.83†	12
160.2	0.82†	13

TWA exposure values are given in mg CS₂/m³ of air.

r = Correlation coefficient found between exposure index E and TWA values.

n = Number of measurements.

* = p > 0.05.

† = p ≤ 0.05.

0.61, $n = 20$ to $r = -0.82$, $n = 13$; $p = 0.05$). When the TTCA results with creatinine concentrations in end of workshift urine samples below 1 mg creatinine per ml and above 3 mg creatinine per ml are also disregarded, a much higher correlation is obtained ($r = 0.84$, $n = 14$, $p = 0.05$). If the increase in TTCA during the workshift is not considered, but only TTCA in the end of workshift urine samples, and only the results from the samples with creatinine concentrations above 1 mg per ml and below 3 mg per ml are accepted a high correlation is also obtained ($r = 0.86$, $n = 13$, $p = 0.05$) (fig 3). Taking into account all the end of workshift urine samples without considering these creatinine limits, the r -value decreases to 0.35, $n = 20$.

The relation between the concentration of TTCA (mmol TTCA per ml urine) in end of workshift urine samples and the logarithm of their discoloration time is shown in fig 4a and indicates the effect of TTCA on the reaction rate between iodine and sodium azide. The correlation between the two is high, $r = -0.92$, $n = 12$, $p = 0.05$. If pure TTCA is dissolved in water and appropriately diluted the discoloration times after adding iodine azide reagent given in fig 4b are obtained. The concentration of TTCA in the pure solution is an exponential function of the discoloration time t —that is, $\text{TTCA} = 125 \cdot 5e^{-0.033t}$, with $r = 0.85$, $n = 6$, $p = 0.05$. The question remains, however, as to whether or not the effect of the CS_2 metabolites on the iodine azide reaction is purely catalytic. Ammoniumglycine dithiocarbamate, a possible metabolite of CS_2 was traced by thin layer chromatography before and

Fig 3 TWA exposure values related to concentration (mmol TTCA per ml) in end of workshift urine samples with creatinine concentrations above 1 mg creatinine per ml and below 3 mg creatinine per ml.

Fig 4 TTCA concentrations in urine (a) (mmol TTCA per ml) and in water (b) related to the logarithm of the discoloration time after adding the iodine azide reagent.

after the iodine azide reaction. The compound was observed to disappear completely after the iodine azide reagent discoloured.

Discussion

There is a strong correlation between the exposure index E calculated according to Vasak's formula for the end of workshift urine samples and the increase in TTCA concentrations during the workshift. Table 2 indicates, however, that the limit of 50 mg CS_2 per m^3 of air which Djuric *et al*⁸ suggested as a threshold cannot be considered to be an absolute cut off point. The correlation between time weighted average atmospheric concentrations of CS_2 obtained by personal monitoring and the increase in urinary TTCA concentration during the workshift can be improved by disregarding the end of workshift urine samples with creatinine concentrations below 1 mg per ml urine and above 3 mg per ml urine. It might have been expected that since the exposure indices E correlate strongly with the TWA exposure values and to the TTCA increments, that the latter would also correlate with the TWA exposure values. That they

do not lies in the fact that the results used to relate the TTCA concentrations to the E values were obtained solely from end of workshift urine samples with creatinine concentrations above 1 mg per ml and below 3 mg per ml urine. Figure 3 indicates that it is possible to collect only end of workshift urine samples to obtain a good estimate of the exposure to CS₂.

The question as to whether or not the determination of TTCA in urine resulting from exposure to 50 mg CS₂ per m³ of air or even below 30 mg CS₂ per m³ of air can be used to evaluate exposure to CS₂ is still to be answered.

As shown in fig 4b, the concentration of TTCA in pure solution after adding iodine azide can be expressed by means of an exponential function, but the concentration of TTCA in urine cannot be expressed by a similar equation. Presumably, the other compounds responsible for the discoloration of the iodine azide reagent in urine have their own specific concentration-discoloration curves, which when added to each other result in a more complicated relationship. As may be seen from fig 4, for a given discoloration time, (log t = 1, for example) more TTCA is necessary in pure solution than in urine to induce the discoloration period, indicating the presence of other catalysing compounds.

It is clear from fig 4 that there is a specific TTCA concentration in urine and water with the same discoloration time. As the TTCA concentration increases, the decrease in discoloration time for the pure solution is not as great as the discoloration time for urine, indicating the presence of other compounds catalysing the iodine azide reaction stronger than TTCA. On the other hand, if the TTCA concentration decreases to 10 mmol per ml, it is obvious that the discoloration time will increase. This increase is greater for urine than for the pure solution indicating a more pronounced effect of the decrease of other compounds on the catalysis of the iodine azide reaction than TTCA.

This research was supported by the Fund for Medical Scientific Research under grant No 3.0013.81.

Requests for reprints to: J Rosier, Laboratorium voor Bromatologie, Farmaceutisch Instituut, Rijksuniversiteit van Gent, Harelbekestraat 72, 9000 Gent, Belgium.

References

- ¹ Vasak V, Venecek M, Kimmelova B. The assessment of the exposure of workers to carbon disulphide by the iodine azide test. *Pracov Lek* 1963;15:145-56.
- ² Feigl F. *Spot tests*. Vol 2. *Organic applications*. Amsterdam; Elsevier Publishing Company, 1954.
- ³ Pergal M, Vukojevic N, Cirin-Popov D, Djuric D, Bojovic T. Carbon disulphide metabolites excreted in the urine of exposed workers. I. Isolation and identification of 2-mercapto-thiazolinone-5. *Arch Environ Health* 1972;25:38-41.
- ⁴ Pergal M, Vukojevic N, Djuric D. Carbon disulphide metabolites excreted in the urine of exposed workers. II. Isolation and identification of thiocarbamide. *Arch Environ Health* 1972;25:42-4.
- ⁵ Van Doorn R, Leijdekkers CM, Henderson P, Vanhoorne M, Vertin P. Determination of thio compounds in urine of workers exposed to carbon disulfide. *Arch Environ Health* 1981;36:289-97.
- ⁶ Rosier J, Vanhoorne M, Grosjean R, Van De Walle E, Billemont G, Van Peteghem C. Preliminary evaluation of urinary 2-thiothiazolidine-4-carboxylic acid (TTCA) levels as a test for exposure to carbon disulfide. *Int Arch Occup Environ Health* 1982;51:159-67.
- ⁷ National Institute for Occupational Safety and Health. *Manual of analytical methods*. Vol 2. 2nd ed. Cincinnati, Ohio: NIOSH, 1977.
- ⁸ Djuric D, Surduick N, Berkes I. Iodine azide test on urine of persons exposed to carbon disulfide. *Br J Ind Med* 1965;22:321-33.
- ⁹ Van Doorn R, Delbressine LPC, Leijdekkers CM, Vertin P, Henderson P. Identification and determination of TTCA in urine of workers exposed to carbon disulfide. *Arch Toxicol* 1981;47:51-8.
- ¹⁰ De Baun JR, Miaullis JB, Knarr J, Mihailowski A, Menn JJ. The fate of N-trichloro (¹⁴C) methylthio-4-cyclohexene-1,2-dicarboximide (¹⁴C-captan) in the rat. *Xenobiotica* 1974;4:101-19.
- ¹¹ Rosier J, Simonds R, Van Peteghem C, Vanhoorne M. The preparation of pure 2-thio-thiazolidine-4-carboxylic-acid (TTCA) as a reference standard for carbon disulfide exposure tests. *Bulletin des Societes Chimiques Belges* 1983;92:397-402.
- ¹² Jakubowski M, Gessner T. S-Methylation of dithiocarbamate: derived from amino acids. *Biochem Pharmacol* 1972;21:3073-6.
- ¹³ National Institute for Occupational Safety and Health. *Summary of NIOSH recommendations for occupational health standards*. Washington DC: US Department of Health and Human Services, 1980.
- ¹⁴ TLV Airborne Contaminants Committee. *Threshold limit value for chemical substances in workroom air adopted by the American Conference of Governmental Industrial Hygienists, Cincinnati*. Cincinnati: ACGIH, 1981.