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

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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 E. 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. to evaluate the degree to which workers are exposed to carbon disulphide. The chemical reaction may be written as:

\[ 2 \text{NaN}_3 + I_2 \rightarrow 3 \text{N}_2 + 2 \text{NaI} \]

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. 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-5 and thiourea, which when added in pure form to the iodine azide solution, discolors 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...
of workers occupationally exposed to CS₂. TTCA is a heterocyclic five membered ring that is formed in the body by the reaction between carbon disulphide and glutathione. The quantification of TTCA in the urine of viscose workers thus seems to have interesting possibilities for the biological monitoring of CS₂ exposure.

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 al8 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.

Urinary 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

A high pressure liquid chromatograph (Spectra-Physics SP 8000) was used, equipped with a Hibar column (250 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 al10 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 workshift 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

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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.
TTCA, b = TTCA concentration in mmol TTCA per mol creatinine; beginning of workshift urine samples.
TTCA, c = TTCA concentration (same units) in end of workshift urine samples.
TTCA, e(a) = TTCA concentration in end of workshift urine samples in 10⁻⁷ 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.
d = No beginning of workshift urine sample available.
f = Discoloration time greater than three hours.

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.

Fig 2 TWA exposure values (in mg CS₂/m³ of air) related to exposure index E of end of workshift urine samples.
Relation between the iodine azide test and the TTCA test for exposure to carbon disulphide

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, TTCA = 125, \( 5e^{-0.03t} \), with \( r = 0.85, n = 6, p = 0.05 \). The question remains, however, as to whether or not the effect of the \( \text{CS}_2 \) metabolites on the iodine azide reaction is purely catalytic. Ammoniumglycine dithiocarbamate, a possible metabolite of \( \text{CS}_2 \) was traced by thin layer chromatography before and 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 \( \text{CS}_2 \) per m³ of air which Djuric et al. suggested as a threshold cannot be considered to be an absolute cut off point. The correlation between time weighted average atmospheric concentrations of \( \text{CS}_2 \) 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.

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