2-Cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile

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ABSTRACT The aim of this study was to evaluate the efficiency of metabolism of acrylonitrile (ACN) to N-acetyl-S-(2-cyanoethyl)-L-cysteine (2-cyanoethylmercapturic acid (CEMA) in man, the kinetics of excretion of this metabolite, and the relation between the uptake of ACN and the excretion of CEMA in urine. Eleven experiments were performed on six male volunteers exposed for eight hours to ACN at concentrations of 5 or 10 mg/m³. The average respiratory retention of ACN was 52% and 21.8% of the retained ACN was excreted as CEMA in urine. Elimination approximated first order kinetics with half life of about eight hours. The best correlation between the uptake of ACN in the lungs and excretion of CEMA in urine was obtained when the concentration of CEMA in the urine fraction, collected between the sixth and eighth hours after the beginning of exposure, was adjusted to a specific gravity of 1.016 ($y = 0.33x - 13.3$; $r = 0.83$). CEMA excretion, however, cannot be used as an individual index of exposure.

According to the International Agency for Research on Cancer there is sufficient evidence for the carcinogenicity of Acrylonitrile (ACN) in animals.¹ Though there is only limited evidence for the carcinogenicity of ACN in man it seemed worth while to investigate the absorption of ACN by all routes.

Acrylonitrile may be absorbed through the respiratory tract (the retention of vapour is 46%) and liquid ACN is absorbed through the skin at the rate of 0.6 mg/cm²/h.² At present, no reliable method for evaluating internal exposure to ACN is available.³

So far attempts aimed at developing such a method have been based on the determinations of thiocyanates and free ACN in urine.^{4,5} High physiological values, evidently induced by smoking,³ interfere with the use of thiocyanates, whereas only 1-4% of the dose is excreted as free ACN as shown by the data of Sakurai *et al*⁴ and Houthuijs *et al*.⁵

Correlations between the concentrations of ACN in the air and excretion of ACN in urine are weak, according to Houthuijs *et al*⁵; correlation coefficients ranged from 0.11 to 0.76 for postshift urine samples and from 0.14 to 0.69 for preshift urine samples. Data from animal experiments obtained by Kopecký *et al*⁶⁻⁹ Langwardt *et al*¹⁰, Ahmed and Patel,¹¹ Van Blanderen *et al*¹², and Sapota and Damiński¹³

showed that CEMA is the main metabolite of ACN and that in rats about 50-60% of the dose is excreted in urine in this form. Therefore, excretion of CEMA seems to have the potential to be an index of internal exposure to ACN.

In preliminary studies Damiński and Trojanowska confirmed the presence of CEMA in the urine of workers engaged in manufacturing acryl fibres (unpublished data). The concentration of CEMA in urine collected at the end of the shift varied between 25 and 350 mg/l. The sensitivity of the method, however, did not allow for the evaluation of the kinetics of CEMA excretion in urine.

The present study aimed to determine the efficiency of ACN metabolism to CEMA, the kinetics of urinary CEMA excretion, and to find a correlation between CEMA excretion in the urine and the absorbed dose of ACN. The study was carried out on volunteers under experimental conditions.

Materials and methods

DETERMINATION OF CEMA IN URINE

To minimise possible errors, we decided to develop two independent methods for determining CEMA in urine and to perform further determinations concurrently in both participating laboratories. Because of their close resemblance only one analytical procedure

(Institute of Occupational Medicine in Łódź) has been described in detail together with basic information concerning both methods.

SYNTHESIS OF CEMA

CEMA was synthesised after Vogel.¹⁴ A white crystalline product was obtained, mp 112–114°C. Analytical calculations for CEMA: C, 44.44%; H, 5.55%; N, 12.96%; S, 14.81%; Found: C, 44.8%; H, 5.86%; N, 11.47%; S, 15.28%.

REAGENTS

- (a) Standard solution of CEMA in ethyl alcohol: 2.5 g/l (11.57 mmol/l).
- (b) Standard solution of N-acetylmethionine (NAM) in ethyl alcohol: 2.5 g/l (13.08 mmol/l).
- (c) Solution of diazomethane in diethyl ether (Vogel¹⁴).
- (d) Ethyl acetate, pa, Polish reagents, Gliwice.
- (e) Sodium chloride, pa, Polish reagents, Gliwice.

GAS CHROMATOGRAPHY

The determinations were carried out with a Varian type 2800 gas chromatograph equipped with an AFID detector. A glass column (1 m × 2 mm id) packed with 3% OV-225 on Varaport 30 (100–120 mesh) was used. The oven temperature was programmed to increase from 140° to 250°C at the rate of 10°C a minute; injector and detector temperatures were 180° and 260°C respectively. Argon was used as a carrier gas at a flow rate of 40 ml/min.

PROCEDURE

To 2.5 ml of urine, 0.1 ml of NAM solution (internal standard) was added. The sample was then acidified with 50 µl of concentrated HCl, saturated with NaCl, and extracted with 25 ml of freshly distilled ethyl acetate for 10 minutes. Then, after centrifugation, the organic layer was evaporated to dryness. The residue was dissolved in 0.25 ml of ethanol and methylated with gaseous diazomethane. One microlitre of aliquot was used for each injection. Concentrations of CEMA were calculated from the ratio of the peak height of CEMA methyl ester and to that of an internal standard after previous calibration.

The second method differed from the first in using an isothermal column temperature of 218°C instead of the programmed temperature increase and in the use of CEMA 1-propyl ester as the internal standard.

Typical chromatograms of the urine extracts are presented in figs 1 and 2. Within the range of concentrations up to 60 mg CEMA/l the relation between the concentrations of CEMA and CEMA/internal standard peak height (method 1) or peak area (method 2) ratios were linear. The detection limits of both methods were about 1 mg CEMA/l urine.

Fig 1 Typical chromatograms of urine extracts: (a) blank, (b) urine of an exposed person containing 15 mg/l of CEMA; ST—internal standard. (Method 1.)

Within-run relative standard deviations ranged from $\pm 11.0\%$ to $\pm 8\%$ (method 1) and from $\pm 8.0\%$ to 3.2% (method 2) for urinary CEMA concentrations of 10–60 and 2–100 mg CEMA/l respectively. Between day imprecision, calculated from six determinations of CEMA solutions in urine during two weeks was within the range from ± 5.0 to $\pm 9.0\%$. Urinary solutions of CEMA kept at $+4^\circ$ and -18°C were stable for at least two weeks.

EXPERIMENTS INTO TOXICOLOGICAL CHAMBER

Subjects

The subjects of this study were six male volunteers aged 28–45 who showed no abnormalities in routine clinical examinations. All the volunteers (including the present authors) were toxicologists fully aware of the toxic properties of ACN.

Exposure conditions

The exposures were carried out in an exposure cham-

Fig 2 Typical chromatograms of blank (a) and spiked urine sample (b) containing 25 mg CEMA/1; ST—internal standard. (Method 2.) × Attenuation reduced 10 fold.

ber at the Institute of Occupational Medicine (IOM) in Łódź. The chamber had a volume of 11.7 m³ and the ventilation was adjusted to about 200 m³/h. ACN vapours were generated in a saturator immersed in the thermostated water bath and diluted with carrier air to produce the desired environmental concentration. ACN concentrations in the chamber were monitored by gas chromatography. The determinations were carried out with a GCHF-18-3 gas chromatograph equipped with an FID detector. A metal column (3.5 m × 4 mm id) packed with 10% Carbowax 20 M on Chromosorb W/AW DMCS was used. The temperatures were as follows: oven 100°C, injector 120°C, detector 180°C. Nitrogen was used as a carrier gas, at the flow rate of 30 ml/min. Five millilitre air samples from the chamber were injected

Table 1 Exposure conditions

Experiment No	No of subjects exposed	Mean concentration of ACN in the chamber		
		mg/m ³	μmol/m ³	s _r
1	4	10.72	202	0.14
2	1	10.7	202	0.09
3	1	10.9	206	0.07
4	1	5.59	105	0.13
5	3	5.81	110	0.05
6	1	5.42	102	0.11

s_r = Relative standard deviation.

directly into the gas chromatograph every 15 minutes with a gas tight Hamilton syringe.

The volunteers were exposed individually or in groups of three or four (table 1) to a constant concentration of ACN approximating to the current Polish MAC value 10 mg/m³ (0.187 mmol/m³) or 5 mg/m³ (0.093 mmol/m³) for eight hours with three 10 minute breaks at two, four, and six hours after the beginning of the experiment. Table 1 gives data on the stability of ACN concentrations in the chamber.

Individual ventilation and retention of ACN in the lungs were measured in separate experiments. A volunteer sat outside the chamber, breathing air supplied from the chamber through a mask. The outlet of the mask was connected by plastic tubing (diameter 3 cm, length 50 cm) to the three necked 21 flask. One outlet of the flask was connected with the respirometer to estimate expired air volumes. The samples of inhaled air were collected directly before the mask and that of expired air from the centre of the flask and concentrations of ACN were determined by means of gas chromatography in conditions described above.

Collection of urine samples

Urine samples were collected after 2, 4, 6, 8, 10, 12, 14, 24, 27, and 31 hours from the start of exposure. The volume of each urine fraction and its creatinine concentration and specific gravity (in temperature about 22°C) were measured. All samples were stored in a refrigerator before the CEMA was determined.

Taking into account the relatively good correlation ($r = 0.95$; $n = 85$) and concordance of the results of determinations performed in both laboratories (IOM = 1.05, Institute of Hygiene and Epidemiology, Prague + 1.5) the mean values of CEMA concentrations in urine are presented.

Results

Retention of ACN in the lungs averaged about 52% (table 2) and about 22% of the retained ACN was metabolised to CEMA (table 3).

The kinetics of excretion of CEMA in urine was not uniform. The maximum rate of excretion occurred at different times after the beginning of exposure. Exam-

Table 2 Retention of ACN in the lungs

Person exposed	ACN conc in chamber (mg/m ³)	Lungs ventilation (l/h)	Retention of ACN			
			Period of observation	No of determinations	Retention	
					Mean	s _r
RR	9.6	508	90 min	8	58	0.09
GP	8.2	423	90 min	8	52	0.19
AS	7.8	366	90 min	8	51	0.08
MP	9.6	625	90 min	8	54	0.09
MJ	10.0	620	8 h	8	44	0.07
IL		353	Not measured, mean value (51.8%) was used for calculations			

S_r = Relative standard deviation.

Table 3 Relation between exposure to ACN and excretion of urinary CEMA

Experiment No	Person exposed	ACN uptake		% of dose excreted in urine as CEMA
		(mg)	(μmol)	
5	AS	7.86	148	20.8
5	GP	9.26	175	20.1
5	RR	12.4	234	14.1
4	MJ	11.0	208	12.8
6	MP	13.3	251	13.8
3	IL	13.9	263	28.9
1	AS	14.5	273	38.7
1	GP	17.1	322	27.5
1	RR	22.9	431	21.2
1	MP	26.2	493	22.5
2	MJ	21.2	398	19.3

21.8%; s_r = 0.35.

ples of the kinetics of CEMA excretion in urine are presented in fig 3.

To evaluate the kinetics of the excretion of CEMA, the mean values from all experiments were used. To eliminate the differences resulting from the absorbed dose depending on the concentration of ACN in the inspired air and individual retention and differences in the metabolism of ACN to CEMA, experimentally obtained excretion rates or concentrations of CEMA in urine were recalculated according to the equation:

$$V_{st} = V_x \times \frac{\bar{S}}{S_x} \times \frac{\bar{W}}{W_x}$$

Where:

V_{st} = standardised rate of excretion (or concentration) of CEMA in urine

V_x = rate of excretion or concentration obtained experimentally

$\bar{S}$ = mean dose of ACN absorbed in all experiments

S_x = dose absorbed during a given experiment

$\bar{W}$ = mean excretion of CEMA in % of absorbed ACN (table 3)

W_x = excretion of CEMA in % of absorbed ACN in the given experiment

The standardised values of the rate of excretion of CEMA or its concentration in the urine are presented in figs 4 and 5. The descending parts of both curves are shown in fig 6. They suggest first order elimination

Fig 3 Examples of kinetics of CEMA excretion in urine.

of CEMA with half life values of about seven and nine hours.

Table 4 gives the relation between the uptake of ACN during experiments and the concentration of CEMA in the urine or its rate of excretion. The best correlation was obtained for the relation between the uptake and concentration of CEMA in fractions of urine collected between 6–8 and 4–8 hours after the beginning of exposure and adjusted to a specific gravity of 1.016 (fig 7).

Fig 4 Mean values of standardised rate of excretion of CEMA in urine. Vertical bars indicate range of obtained values. x ---- x diuresis.

Relative standard deviations of experimentally obtained points from the regression lines amounted to $\pm 29\%$ for the urine fraction collected between six and eight hours after the beginning of exposure and $\pm 33\%$ for the fraction collected between four and eight hours. Up to eight hours—that is, during exposure—there seemed to be some relation between the urinary excretion of CEMA and urine flow (fig 4). Correlation between the dose of ACN and the rate of excretion of CEMA was significant only when excretion was expressed for a 24 hour period (table 4). Correlations between the absorbed dose of ACN and concentration of CEMA in urine corrected to the creatinine concentration were insignificant (table 4).

Discussion

A single eight hour experimental inhalational exposure to ACN at concentrations of 5–10 mg/m³ produced no subjective symptoms such as headache, nausea, or general weakness described at a similar level of industrial exposure.¹⁵

The retention of ACN in the lungs, averaging 52%, was similar to the value of 46% reported by Rogaczewska and Piotrowski.² It is interesting that the same subject exposed in 1968 had a retention of 49% and during the present experiment 44%. It indicates that retention of acrylonitrile is constant through many years.

Because of the observed individual differences in the kinetics of CEMA excretion and relatively poor correlation between the uptake of ACN and concentration of CEMA in the urine (even when corrected to specific gravity (sg) = 1.016), this test cannot be ap-

Fig 5 Mean values of standardised concentrations of CEMA in urine (sg corrected to 1.016). Vertical bars indicate range of obtained values.

plied as an individual index of exposure. It seems that it would be more reliable to accept the upper limit of CEMA concentration in urine corresponding to a given concentration of ACN in air. Such an index would be useful in monitoring unexpectedly high absorption—for example, through the skin.

The dose, which can be absorbed at the admissible concentration of ACN in the air, may be calculated according to the formula:

$$D = C \times R \times V$$

where:

C—ACN concentration in the air

R—ACN retention in the lungs (about 50%)

V—lungs ventilation (about 7 m³/8 h)

Fig 6 Descending parts of excretion curves of CEMA in urine.

Table 4 Correlation between absorbed dose of ACM and excretion of CEMA in urine

Urine fraction	Relation between ACN uptake (μmol) and:	No	Regression equation	r	p
4-8 h from beginning of exposure	CEMA conc ($\mu\text{mol/l}$; sg corrected to 1.016)	10	CEMA = 0.285 ACN - 6.68	0.78	<0.01
	CEMA conc ($\mu\text{mol/mmol creat}$)	10	CEMA = 0.012 ACN + 1.35	0.56	NS
	Rate of CEMA excretion ($\mu\text{mol/h}$)	11	CEMA = 0.012 ACN + 0.56	0.60	NS
6-8 h from beginning of exposure	CEMA conc ($\mu\text{mol/l}$; sg corrected to 1.016)	10	CEMA = 0.333 ACN - 13.3	0.83	<0.01
	CEMA conc ($\mu\text{mol/mmol creat}$)	11	CEMA = 0.014 ACN + 1.84	0.42	NS
	Rate of CEMA excretion ($\mu\text{mol/h}$)	11	CEMA = 0.014 ACN + 0.98	0.49	NS
0-24 h from beginning of exposure	Rate of CEMA excretion ($\mu\text{mol/24 h}$)	11	CEMA = 0.197 ACN + 0.27	0.73	<0.01

The dose corresponding to the United States TLV (4.5 mg/m^3) and the proposed Polish MAC value (2 mg/m^3) amounts to 15.7 mg ($290 \mu\text{mol/l}$) and 7 mg ($130 \mu\text{mol/l}$) respectively. These two values are within the range covered by the test (fig 7).

On the basis of the regression curve (fig 7) and the relative standard deviation of the values obtained experimentally amounting to $\pm 29\%$ one may calculate the mean values of CEMA concentrations in the urine which are equivalent to ACN concentrations in air and their fiducial ranges¹⁶ in 90% of samples (mean $\pm 1.8 \text{ SD}$).

Such values would amount to:

- $84 \mu\text{mol/l}$ ($40\text{--}128 \mu\text{mol/l}$) for the United States TLV 4.5 mg/m^3
- $31 \mu\text{mol/l}$ ($15\text{--}47 \mu\text{mol/l}$) for the proposed Polish MAC value 2 mg/m^3 .

Fig 7 Relation between ACN uptake and concentration of CEMA in urine fractions collected between six and eight hours after beginning of exposure (sg of urine corrected to 1.016).

Any CEMA concentration in urine (sg corrected to 1.016) lower than $40 \mu\text{mol/l}$ would mean that ACN absorption was below the United States TLV. Concentrations greater than $130 \mu\text{mol/l}$ strongly suggest that the uptake of ACN was higher than the permissible exposure.

As the half life of CEMA excretion in urine was about eight hours, one can predict the possibility of slight, approximately 10%, increase of CEMA concentration in urine collected on the second day of exposure.

The described method should be evaluated under industrial conditions.

We thank our colleagues for participating in the experiments, Mrs I Hołub for synthesis of CEMA at the IOM, Łódź, Mrs L Heroldová and Mrs E Jabłońska for excellent technical help, and Mr A Olczyk for technical operation of the chamber.

This study was performed within the framework of UNDP supported project WHO/EURO/RER/82/002.

References

- International Agency for Research on Cancer. *Monographs on the evaluation of carcinogenic risk of chemical to humans* Lyon: IARC, 1982: suppl 4.
- Rogazewska T, Piotrowski J. Experimental evaluation of the absorption routes of acrylonitrile in humans. *Med Pracy* 1968;19:349-54.
- Bertelli G, Berlin A, Roi R, Alessio L. Acrylonitrile. In: Alessio L, Berlin A, Boni M, Roi R, eds. *Biological indicators for the assessment of human exposure to industrial chemicals*. Luxembourg: Commission of the European Communities, Industrial Health and Safety, 1984:5-13.
- Sakurai H, Onodema M, Utsunomiya T, Minakuchi H, Iwani H, Matymumura H. Health effects of acrylonitrile in acrylic fibre factories. *Br J Ind Med* 1978;35:219-25.
- Houthuijs D, Remijn B, Willems H, Boleij J, Bierstecker K.

- Biological monitoring of acrylonitrile exposure. *Am J Ind Med* 1982;3:313–20.
- 6 Kopecký J, Gut I, Nerudová J, Zachradová D, Holéček V. Two routes of acrylonitrile metabolism. *J Hyg Epidemiol Microbiol Immunol* 1981;24:356–62.
 - 7 Kopecký J, Gut I, Nerudová J, Zachradová W, Holéček V, Filip J. Acrylonitrile metabolism in the rat. *Arch Toxicol* 1980; suppl 4:322–4.
 - 8 Kopecký J, Zachradová D, Gut I, Filip J. Acrylonitrile metabolism in the rat in vivo. *Pracovny Lekarstvi* 1979;31:203–9.
 - 9 Kopecký J, Gut I, Nerudová J, Zachradová D, Holéček V. Metabolic studies on acrylonitrile. In: Gut I, Cikrt M, Plas GL, eds. *Industrial and environmental xenobiotics*. Berlin: Springer Verlag, 1981:221–30.
 - 10 Langwardt PW, Putzig CL, Braun WH, Young JB. Identification of the major urinary metabolites of acrylonitrile in the rat. *J Toxicol Environ Health* 1980;6:173–282.
 - 11 Ahmed AE, Patel K. Studies on the metabolism of aliphatic nitriles. *Toxicol Appl Pharmacol* 1979;48:A91.
 - 12 Van Blanderer PJ, Delbressine LPC, Hoogeterp JJ, et al. Formation of mercapturic acids from acrylonitrile, crotonitrile, and cinnamonnitrile by direct conjugation and via an intermediate oxidation process. *Drug Metab Dispos* 1981;91:246–9.
 - 13 Sapota A, Dramiński W. The fate of ^{14}C acrylonitrile in rats. In: Gut I, Cikrt M, Plas GL, eds. *Industrial and environmental xenobiotics*. Berlin: Springer-Verlag, 1981:231–7.
 - 14 Vogel AJ. *Textbook of Practical Chemistry*. London: Longmans, 1956.
 - 15 Babanov GP, Kljucikov VN, Karajeva NJ, Liljeva ZV. Clinical symptoms of chronic poisoning by acrylonitrile. *Vrach Delo* 1959;8:833–6.
 - 16 Imamura T, Ikeda M. Lower fiducial limit of urinary metabolite level as an index of excessive exposure to industrial chemicals. *Br J Ind Med* 1973;30:289–92.