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Ethoxyacetic acid: a metabolite of ethylene glycol monoethyl ether acetate in man

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ABSTRACT Urinary excretion of ethoxyacetic acid during and after exposure to ethylene glycol monoethyl ether acetate (EGEE-Ac) was followed up in ten healthy male volunteers. During exposure to EGEE-Ac, ethoxyacetic acid levels appeared with a half life of 2·3 ± 0·1 h. Ethoxyacetic acid excretion continued to increase after exposure was discontinued reaching maximal levels three to four hours later. The decline afterwards could generally be described assuming a half life of 23·6 ± 1·8 h. A second maximum excretion of ethoxyacetic acid, however, was noticed about three hours after the first. Redistribution of EGEE-Ac or ethoxyacetic acid, or both, from a peripheral compartment to the central compartment could explain this observation. Ethoxyacetic acid excretion increased with an increase in the uptake of EGEE-Ac due to higher exposure concentrations or pulmonary ventilation rate during physical exercise. On average 22·2 ± 0·9% of the absorbed EGEE-Ac was recovered within 42 hours. Recovery did not change with a higher intake of EGEE-Ac. At any time after the exposure, quantitative relations between ethoxyacetic acid excretion rate and absorbed dose of EGEE-Ac were found. Monitoring ethoxyacetic acid excretion may therefore be used as a measure of a single exposure to EGEE-Ac.

When animals were exposed to ethylene glycol ethers, the respective alkoxyacetic acid was identified as the major urinary metabolite.[1-6] Using ^{14}C-labelled ethylene glycol ethers, 60–80% of the radioactivity was recovered in the urine of exposed animals. When rats were exposed to unlabelled ethylene glycol monoethyl ether (EGEE), however, the combined excretion of free and conjugated ethoxyacetic acid was only 30% of the absorbed dose.[4]

Recently, ethoxyacetic acid has been detected in the urine of man during and after exposure to EGEE.[7,8] On average 23% of the absorbed EGEE was recovered in the urine within 42 hours. Compared with rats and dogs,[9] the biological half life of ethoxyacetic acid excretion was 2·5–3 times greater in man. At any time after the exposure period, ethoxyacetic acid excretion was found to be proportional to the absorbed dose of EGEE.

Since the ethyl ether of ethylene glycol acetate (EGEE-Ac) is believed to pass through the same metabolic pathway as EGEE after hydrolysis of the ester moiety, similar pharmacokinetics of ethoxyacetic acid excretion are to be expected. To confirm this, the urinary excretion of ethoxyacetic acid was studied in man during and after exposure to EGEE-Ac.

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Subjects and methods

SUBJECTS AND EXPOSURE CONDITIONS

Ten male volunteers (aged 21–30) were exposed to ethylene glycol monoethyl ether acetate (EGEE-Ac) after full information and a written consent. Five were exposed to 14 mg/m^3, 28 mg/m^3, and 50 mg/m^3 EGEE-Ac at rest and five to 28 mg/m^3 EGEE-Ac at rest and during standard physical exercise at 30 W and 60 W.

The exposure period was four hours with a short break of 10 minutes at the end of each hour to allow for urine collection. Between the experiments, the subjects remained unexposed for at least one week (usually two or three weeks). Alcohol consumption was forbidden from the evening before the experiment till after the last urine collection to avoid interference with ethoxyacetic acid formation.

MEASUREMENTS AND CALCULATIONS

Samples of inspired and mixed expired air were taken and analysed as described elsewhere.[10] The time-weighted uptake was calculated from EGEE-Ac con-

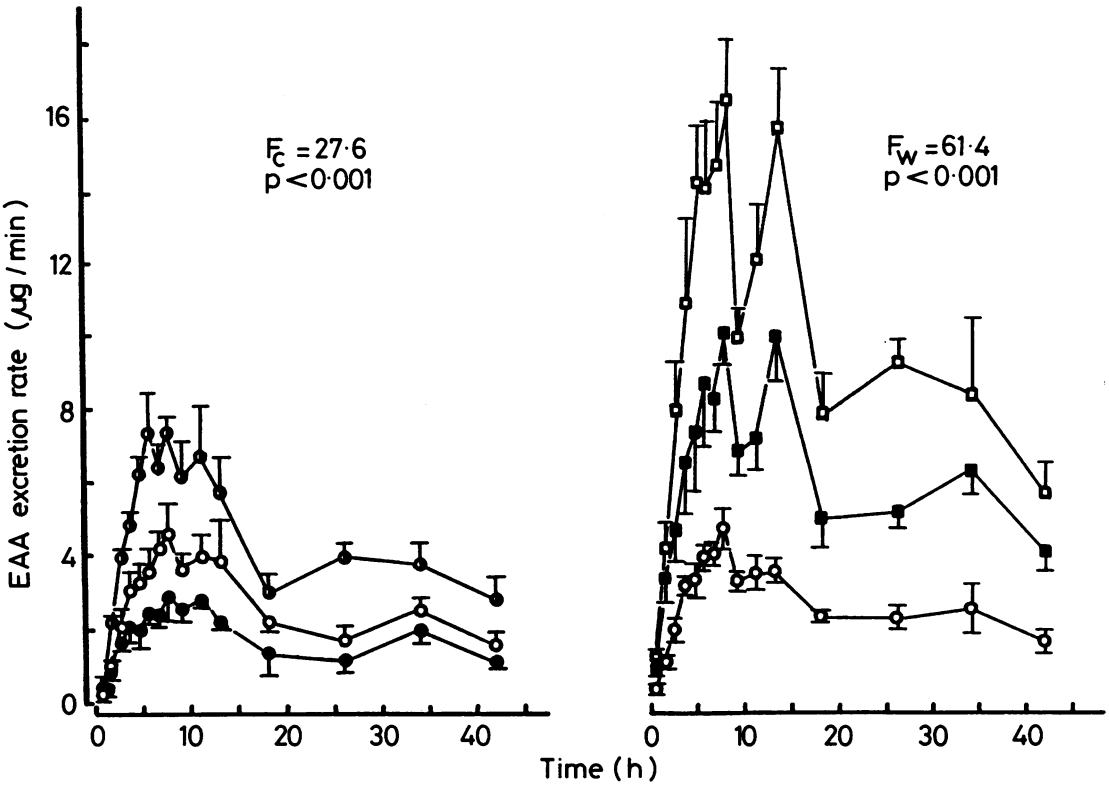

Fig 1 *Urinary excretion of ethoxyacetic acid during and after a four hour exposure to EGEE-Ac under various conditions: 14 mg/m³ (●), 28 mg/m³ (○), and 50 mg/m³ (◑) at rest or 28 mg/m³ at 30 W (■) and 60 W (□). Data are means ± SEM for five subjects. Statistical data are F ratios from three way ANOVA: c = exposure concentration, w = workload.*

centrations of inhaled (C_I-mg/l) and exhaled (C_E-mg/l) air, pulmonary ventilation rate ($\dot{V}_E$-l/min), and exposure time (min):

$$\text{Uptake (mg)} = (C_I\text{-}C_E).\dot{V}_E.\text{time}.$$

Urine samples were taken just before the exposure period and at every hour from the start until the fourth hour after exposure. Urine collections were continued until the morning of the second day after the exposure period (3 × 2 h collections and 4 × 8 h collections). On each sample, urinary volume, density, and creatinine concentration were assessed. The urine samples were analysed gaschromatographically for the presence of ethoxyacetic acid as described by Groeseneken *et al.*[7]

STATISTICAL ANALYSIS

Data were analysed using three way ANOVA with experimental condition, collection time, and subjects as main sources of variation. Single and multiple linear regression analyses were used where appropriate.

Results

URINARY EXCRETION OF ETHOXYACETIC ACID

After one hour of exposure to EGEE-Ac ethoxyacetic acid was detected in the urine. With all experimental conditions, the excretion rate of ethoxyacetic acid increased as exposure proceeded; the half life of appearance was 2·3 ± 0·1 h. A maximal excretion rate was reached three to four hours after exposure was discontinued (fig 1). The slow decrease afterwards could generally be described assuming a biological half life of 23·6 ± 1·8 h. When the time course of the excretion rate was studied in more detail, however, a second maximum could be identified about three hours after the first. This was especially pronounced after exposure during physical exercise. A similar time course was obtained when the excretion rate was expressed per gram of creatinine.

The ethoxyacetic acid excretion rate was higher as the uptake of EGEE-Ac increased as a consequence of higher exposure concentrations (F_c = 27·6; p <

Table 1 *EGEE-Ac uptake and ethoxyacetic acid (EAA) excretion. (Data are means ± SEM for five subjects)*

Experimental conditions	*EGEE-Ac absorbed (mg)*	*EAA excreted in 42 h (mg-equiv of EGEE-Ac)*	*% Recovered in urine*
Group 1:			
14 mg/m³—0 W	23·3 ± 2·1	5·34 ± 0·64	22·9 ± 1·3
28 mg/m³—0 W	44·9 ± 1·3	8·76 ± 0·88	19·7 ± 2·4
50 mg/m³—0 W	85·1 ± 5·5	15·47 ± 0·68	18·3 ± 0·7
	F = 128·2	F = 38·6	F = 3·09
	p < 0·001	p < 0·001	NS
Group 2:			
28 mg/m³—0 W	37·1 ± 2·4	8·77 ± 0·85	23·2 ± 1·6
28 mg/m³—30 W	84·4 ± 2·5	19·94 ± 1·86	23·5 ± 1·9
28 mg/m³—60 W	121·5 ± 5·4	30·83 ± 3·32	25·9 ± 3·4
	F = 146·7	F = 59·0	F = 1·0
	p < 0·001	p < 0·001	NS

0·001) or of increasing workload during physical exercise (F_w = 61·4; p < 0·001), the latter being primarily due to an increased pulmonary ventilation rate (F = 129·7; p < 0·001).

RECOVERY OF ETHOXYACETIC ACID

Ethoxyacetic acid excretion was monitored up to 42 hours after the start of exposure. The total amount excreted was calculated by trapezoidal integration of the excretion curves and expressed as mg-equivalent of EGEE-Ac (table 1).

The 42 hour excretion of ethoxyacetic acid increased proportionally to the time weighted uptake of EGEE-Ac (F = 38·6; p < 0·001 for exposure to in-

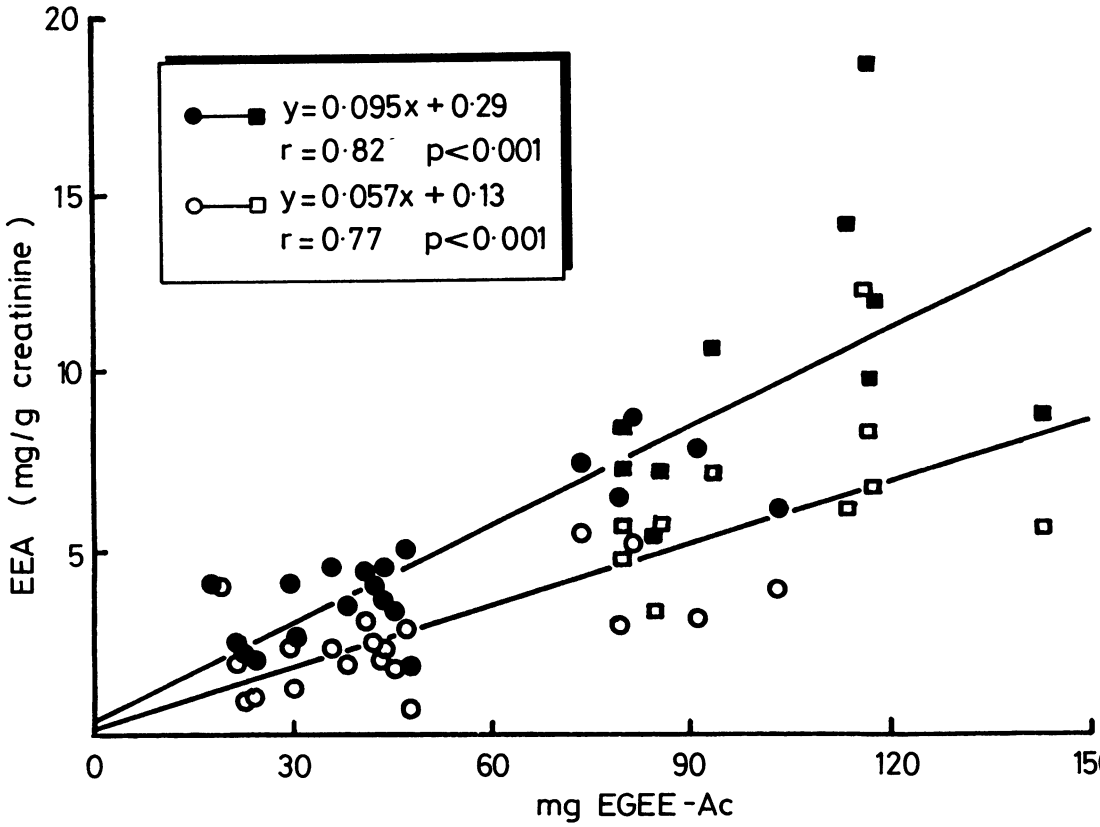

Fig 2 *Relation between time weighted uptake of EGEE-Ac at rest (○, ●) and during physical exercise (□, ■), and urinary excretion rate of ethoxyacetic acid at maximal excretion (closed symbols) and on morning of next day (open symbols).*

creasing concentrations and $F = 59{\cdot}0$; $p < 0{\cdot}001$ for exposure during physical exercise). The amount of EGEE-Ac recovered in the urine as ethoxyacetic acid within 42 hours averaged $22{\cdot}2 \pm 0{\cdot}9\%$. Recovery did not change with any experimental condition ($F \leqslant 3{\cdot}6$; NS).

SUBJECT RELATED FACTORS AFFECTING ETHOXYACETIC ACID EXCRETION

In single regression analysis the amount of ethoxyacetic acid excreted within 42 hours was related to the EGEE-Ac concentration in the inspired air ($r = 0{\cdot}19$; $p < 0{\cdot}05$) and the uptake rate ($r = 0{\cdot}84$; $p < 0{\cdot}001$). Cardiopulmonary variables such as ventilation rate ($r = 0{\cdot}83$; $p < 0{\cdot}001$), oxygen consumption ($r = 0{\cdot}83$; $p < 0{\cdot}001$), respiratory frequency ($r = 0{\cdot}24$; $p < 0{\cdot}01$), and heart rate ($r = 0{\cdot}87$; $p < 0{\cdot}001$) were also significant contributory factors to ethoxyacetic acid excretion. Furthermore, ethoxyacetic acid correlated positively with the percentage body fat ($r = 0{\cdot}40$; $p < 0{\cdot}001$) and negatively with height ($r = -0{\cdot}17$; $p < 0{\cdot}05$). No correlation was found with body weight.

In a multiple linear regression model using a step up procedure the correlation with inhaled concentration and pulmonary ventilation rate did not persist when respiratory uptake was incorporated in the model. In addition to uptake rate ($r = 0{\cdot}69$; $p < 0{\cdot}001$) factors determining transport to the metabolic compartment (heart rate: $r = 0{\cdot}77$; $p < 0{\cdot}001$) and indices of distribution volume (height: $r = 0{\cdot}55$; $p < 0{\cdot}001$ and percentage body fat: $r = 0{\cdot}60$; $p < 0{\cdot}001$) contributed significantly to ethoxyacetic acid excretion.

ETHOXYACETIC ACID EXCRETION AS AN INDEX OF EGEE-AC EXPOSURE

Ethoxyacetic acid excretion was found to increase proportionally with higher uptake rates of EGEE-Ac. Furthermore, the recovery of ethoxyacetic acid within 42 hours did not differ with any of the experimental conditions used. These observations suggest that a constant fraction of EGEE-Ac is metabolised to, and excreted as, ethoxyacetic acid. Ethoxyacetic acid in urine samples after the exposure period may therefore serve as an index of EGEE-Ac exposure.

At any time up to 42 hours after the exposure period, good correlations ($r \geqslant 0{\cdot}76$; $p < 0{\cdot}001$) were observed between the ethoxyacetic acid excretion rate and the time weighted uptake of EGEE-Ac. Similar correlations were found when ethoxyacetic acid excretion was expressed in mg/g creatinine (fig 2). Biological exposure indices may be derived from a single exposure. Urinary concentration corrected for a mean urine density of 1·024 and excretion rate of ethoxyacetic acid are presented in table 2 at three different times after exposure under the various experimental conditions.

Discussion

During and after exposure to EGEE-Ac, ethoxyacetic acid was excreted in the urine as was observed with exposure to EGEE.[8] These findings confirm the hypothesis that EGEE-Ac is first converted to EGEE by esterases and passes through the same metabolic pathway afterwards.[9]

The time course of ethoxyacetic acid excretion during and after exposure to EGEE-Ac is similar to that

Table 2 *Urinary concentrations and excretion rates of ethoxyacetic acid after exposure to EGEE-Ac. (Data are means ± SEM)*

Experimental conditions	*Time after end of exposure (h)*	*Concentration† (mg/l)*	*Excretion rate*	
			(μg/min)	*(mg/g creatinine)*
14 mg/m³—0 W (n = 5)	0	1·89 ± 0·49	2·08 ± 0·41	2·35 ± 0·50
	4	2·20 ± 0·30	2·89 ± 0·63	2·59 ± 0·39
	18*	1·97 ± 0·76	1·35 ± 0·39	1·81 ± 0·60
28 mg/m³—0 W (n = 10)	0	3·50 ± 0·23	3·11 ± 0·28	3·20 ± 0·35
	4	4·04 ± 0·52	4·65 ± 0·46	3·89 ± 0·28
	18*	2·57 ± 0·24	2·29 ± 0·34	2·12 ± 0·20
50 mg/m³—0 W (n = 5)	0	4·57 ± 0·46	4·78 ± 0·42	5·87 ± 0·57
	4	6·46 ± 1·01	7·36 ± 0·42	7·29 ± 0·45
	18*	5·61 ± 0·85	3·01 ± 0·51	4·15 ± 0·52
28 mg/m³—30 W (n = 5)	0	4·76 ± 0·87	6·43 ± 1·32	6·04 ± 1·45
	4	7·29 ± 1·01	9·97 ± 0·38	7·80 ± 0·88
	18*	5·57 ± 0·85	5·01 ± 0·79	5·32 ± 0·62
28 mg/m³—60 W (n = 5)	0	8·63 ± 1·31	10·75 ± 2·36	9·82 ± 2·23
	4	13·72 ± 1·13	16·29 ± 1·55	12·76 ± 1·78
	18*	9·27 ± 1·20	7·75 ± 1·08	7·78 ± 1·21

*Morning collection of the next day.
†Corrected for a mean urine density of 1·024.

found after EGEE exposure.[8] Maximal excretion rate was reached three to four hours after the end of the exposure period. At that time the respiratory elimination of both unmetabolised EGEE[10] and EGEE-Ac[11] was almost negligible, attaining respectively 7·0% and 10·6% of the values measured 7·5 minutes after exposure. The calculated half life of ethoxyacetic acid elimination was 23·6 ± 1·8 h as was the case after exposure to EGEE.[8] The additional hydrolysis of the ester moiety of EGEE-Ac could therefore not be a rate limiting step in the pharmacokinetics of EGEE-Ac, since its estimated half life is only 9–12 minutes.[9 11]

The observation that ethoxyacetic acid excretion also showed a second maximum about three hours after the first was unexpected; it was especially pronounced after exposure during physical exercise. In the case of exposure to EGEE no such time course of ethoxyacetic acid excretion was noticed.[8] After revision of the data, however, the second maximum may also be present, although less pronounced. This phenomenon could be attributed to a redistribution of the glycol ethers or the metabolite, or both, from a peripheral to the central compartment (G Mannaerts, personal communication). More detailed information on such a mechanism might be offered by plasma concentrations of both the glycol ethers and ethoxyacetic acid. The available method for glycol ether determination in plasma,[12] however, is not sensitive enough for concentrations as low as those used in these experimental conditions. Furthermore, methods to assess plasma concentrations of ethoxyacetic acid are lacking. The occurrence of such a redistribution mechanism might explain the large differences in the elimination half life between man and animal models: compared with rats[2] and dogs,[9] the half life of urinary ethoxyacetic acid excretion is 2·5–3 times greater in man.

The urinary excretion of ethoxyacetic acid after exposure to EGEE-Ac seemed to be governed mainly by EGEE-Ac uptake rate and indices of transport mechanisms to the metabolic compartment and of distribution volume. Within 42 hours, on average, 22·2% of EGEE-Ac absorbed was metabolised and excreted in the urine as ethoxyacetic acid. This observation is in agreement with the results of the EGEE exposure study,[8] indicating that EGEE-Ac is metabolised in man to the same extent as EGEE. By contrast, 60–80% of absorbed ethylene glycol ethers were recovered in the urine of animals.[1–3 5 6] Only Jönsson *et al* estimated recovery in rats to be about 30% of the absorbed dose.[4]

As part of the health monitoring of workers exposed to EGEE-Ac, urinary excretion of ethoxyacetic acid may be used as a measure of exposure. At any time after the exposure period, ethoxyacetic acid excretion rate correlated with the time weighted uptake of EGEE-Ac as was also observed in the case of EGEE.[8] Moreover, for equivalent doses of EGEE-Ac or EGEE absorbed, the same amount of ethoxyacetic acid is excreted. As a consequence, the same relationship between ethoxyacetic acid excretion and time weighted uptake of EGEE or EGEE-Ac (expressed as EGEE-equivalents) is found by analysis of covariance (F = 0·56; NS for data at maximal excretion rate):

EAA (mg/g creat) = −0·75 + 0·152 mg EGEE[8]

EAA (mg/g creat) = 0·29 + 0·140 mg EGEEeq (this study)

These findings must, however, be treated with care. The biological exposure indices which may be derived from these experiments are probably valid only for a single exposure to EGEE or EGEE-Ac, since accumulation of ethoxyacetic acid may occur through repetitive exposures as a consequence of the long biological half life of elimination.

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