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RESEARCH ARTICLE

Biomarkers of the alcohol hangover state: Ethyl glucuronide (EtG) and ethyl sulfate (EtS)

Marlou Mackus[1] | Aurora J. A. E. van de Loo[1,2] | S. Jorinde Raasveld[1] | Anna Hogewoning[1] | Javier Sastre Toraño[3] | Frits M. Flesch[3] | Gerdien A. H. Korte-Bouws[1] | Renier H. P. van Neer[3] | Xiaochun Wang[3] | Thomas T. Nguyen[3] | Karel A. Brookhuis[4] | Aletta D. Kraneveld[1,2] | Johan Garssen[1,5] | Joris C. Verster[1,2,6]

[1] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands

[2] Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands

[3] Division of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands

[4] Faculty of Behavioral and Social Sciences, Groningen University, Groningen, The Netherlands

[5] Nutricia Research, Utrecht, The Netherlands

[6] Centre for Human Psychopharmacology, Swinburne University, Melbourne, VIC, Australia

Correspondence
Joris C Verster, Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584CG Utrecht, The Netherlands.
Email: j.c.verster@uu.nl

Abstract

Introduction The aim of this study was to investigate the usefulness of ethyl glucuronide (EtG) and ethyl sulfate (EtS) as biomarkers of the hangover state.

Methods Thirty-sixhealthy social drinkers participated in this study, being of naturalistic design. Eighteen participants experience regular hangovers (the hangover group), whereas the other 18 claim to not experience a hangover (the hangover-immune group). On a control day (alcohol-free) day and a post-alcohol day, urine EtG and EtS concentrations were determined and hangover severity assessed.

Results Urinary EtG and EtS concentrations were significantly increased on post-alcohol day compared to the control day (p = .0001). Both EtG and EtS concentrations did not significantly correlate with the overall hangover score, nor with the estimated peak blood alcohol concentrations and number of alcoholic drinks. EtG correlated significantly only with the individual hangover symptom "headache" (p = .033; r = .403). No significant correlations were found with the EtG to EtS ratio. EtG and EtS concentrations significantly correlated with urine ethanol concentrations.

Conclusions Although urine EtG and EtS concentration did not significantly correlate to estimated peak blood alcohol concentrations or the number of alcoholic drinks consumed, a significant correlation was found with urine ethanol concentration. However, urine EtG and EtS concentrations did not significantly correlate with overall hangover severity.

KEYWORDS

alcohol, biomarker, EtG, ethanol, EtS, hangover

1 | INTRODUCTION

Alcohol is the most abused drug worldwide, and hangovers are the most commonly reported adverse consequence of excessive alcohol consumption. A hangover refers to the combination of mental and physical symptoms, experienced the day after a single episode of heavy drinking when the blood alcohol concentrations approaches zero (van Schrojenstein, Mackus, van de Loo, & Verster, 2016).

Hangovers do not only have a negative effect on one's health and well-being, but also have socioeconomic consequences, such as missing appointments and poor performance at work (Verster & Frone 2015). For instance, more than half of Dutch long-haul truck drivers report driving during the hangover state while they acknowledge that their driving is less safe and less responsible compared to nondrinking days (Verster, van der Maarel, McKinney, Olivier, & de Haan, 2014). This was confirmed by an experimental study that demonstrated

significant driving impairment during alcohol hangover (Verster, Bervoets, et al., 2014). Of note, the magnitude of driving impairment was comparable to that observed after consuming alcohol to reach a blood alcohol concentrations (BAC) of 0.05%, that is, the legal limit for driving in many countries (Holloway, 1994). Given the potential risks of being hungover, it is important to identify suitable biomarkers for the hangover state. At this moment, aside from self-report, it is not possible to easily identify people who are suffering from a hangover. Breath alcohol test readings are likely to be zero (Stephens, Grange, Jones, & Owen, 2014). Preferably, such a biomarker should be easy to measure (i.e., quick, noninvasive assessments), with a direct relationship between its concentration in blood, urine, breath, or saliva and hangover severity, or the magnitude of performance impairment observed during hangover.

Previous research suggested a potential role for the minor nonoxidative metabolites ethyl glucuronide (EtG) and ethyl sulfate (EtS) as a biomarker of the alcohol hangover state (Hoiseth, Fosen, Liane, Gostrand, & Morland, 2015; Hoiseth et al., 2008; Smith & Dischinger, 2010; Stephens et al., 2014). EtG is a nonoxidative minor metabolite of ethanol, formed by the process of glucuronidation, which is catalyzed by UDP-glucuronosyltransferase (Foti & Fisher, 2005). EtS is another nonoxidative metabolite of ethanol, formed by sulfate conjugation through the action of cytosolic sulfotransferase (Helander & Beck, 2005; Wurst et al., 2006). Both EtG and EtS can be determined in blood, urine, and saliva.

Although the formation of EtG and EtS only represents 0.1% of total alcohol metabolism, both metabolites can already be determined after consumption of relative small amounts of ethanol (Hoiseth et al., 2008). In urine, EtG and EtS are detectable in urine up to 35 hr after alcohol consumption, opening a detection window that includes the occurrence of hangover symptoms (Dahl, Stephanson, Beck, & Helander, 2002; Schmitt, Aderjan, Keller, & Wu, 1995). Therefore, even if ethanol is no longer detectable in breath, recent alcohol consumption can still be demonstrated by the presence of EtG and EtS (Helander & Beck, 2005).

Up to now, research on the possible relationship of EtG and EtS concentration and hangover severity is limited. Hoiseth et al. (2015) investigated the prevalence of hungover drivers and corresponding concentrations of EtG and EtS in blood. Out of 146 cases, 90 of the drivers were judged to be impaired in their driving. In only 16 of these 90 cases of impaired driving, blood samples were tested positive for both EtG and EtS. Nevertheless, concentrations of EtG and EtS significantly correlated to the degree of impairment. Another study investigating injured patients after alcohol consumption revealed that in 17% of them, EtG and EtS could be detected in their blood, despite their BAC having returned to zero. However, in this study, no significant correlation was found between EtG and EtS concentrations, hangover severity, and the magnitude of driving impairment (Bogstrand, Hoiseth, Rossow, Normann, & Ekeberg, 2014). Neumann et al. (2008) determined blood EtG concentration to demonstrate recent alcohol use of emergency room patients. Blood samples from minimally injured and clinically nonintoxicated patients were collected. Although 38% of these patients tested positive for EtG, their blood EtG concentration did not correlate significantly with their scores on the Alcohol Use Disorders Identification Test (AUDIT).

In summary, inconclusive results have been presented regarding the association of EtG/EtS concentration and hangover severity, alcohol consumption outcomes, and performance impairment. Therefore, the aim of this study was to further examine the relationship between EtG and EtS concentrations and hangover severity. Whereas previous studies determined EtG and EtS in blood, this study assessed these metabolites in urine, as this noninvasive method of data collection is less of a burden to participants.

2 | METHODS

Social drinkers (N = 36), 18–30 years old, who reported to consume at least five alcoholic beverages per occasion, at least three times per month, were recruited. To be included, subjects had to be healthy volunteers that consume sufficient amounts of alcohol to produce an alcohol hangover. The latter was defined as having an estimated peak BAC % (wt/vol) of at least 0.08%, as the majority of drinkers report having a hangover when exceeding this BAC level (Verster, de Klerk, Bervoets, & Kruisselbrink, 2013). This was determined by asking subjects how many alcoholic drinks they usually consume within a certain time frame. In the Netherlands, a standardized alcoholic consumption contains 10 g of ethanol. Their estimated peak BAC was computed using Watson, Watson, and Batt (1981) formula, which considers drinking time and amount of alcohol consumed, and controls for gender and body weight. Recreational drug users and smokers were excluded from participation in the study. N = 18 subjects who report having hangovers, and N = 18 subjects who report not to have hangovers after heavy alcohol consumption (i.e., having an estimated peak BAC > 0.08%) participated in the study. The University of Groningen Psychology Ethics Committee approved the study, and written informed consent was obtained from all participants.

The study consisted of a post-alcohol day and an alcohol-free control day. In the evening, subjects consumed alcohol in a setting of their own choice. In this so-called naturalistic study design, the investigators are not present during the drinking session and no constraints were posed on their (drinking) behavior. Participants were however instructed not to use recreational drugs or smoke cigarettes. They were asked not to consume any alcoholic nor caffeinated beverages 24 hr prior to the alcohol-free control day.

On test days, subjects came to the institute in the morning. Urine samples were collected at 09.30 a.m. In addition, several subjective assessments were made (for a detailed description, see Hogewoning et al., 2016). Subjects reported the number and type of alcoholic beverages that were consumed the evening before the test day and the duration of alcohol consumption. This allowed calculating their estimated peak BAC (Watson et al., 1981). A 1-item overall hangover severity score, and the severity score of 23 individual hangover symptoms were rated on a 11-point scale, ranging from 0 (*absent*) to 10 (*extreme*; Hogewoning et al., 2016). The 23 individual-assessed hangover symptoms were headache, nausea, concentration problems, regret, sleepiness, heart beating, vomiting, tiredness, shaking, clumsiness, weakness, dizziness, apathy, sweating, stomach pain, confusion, light sensitivity, thirst, heart racing, anxiety, depression, reduced appetite, and sleep problems. The AUDIT was completed to identify

drinkers with a hazardous and harmful pattern of alcohol consumption (Saunders, Aasland, Babor, de la Fuente, & Grant, 1993), and the Self Rating of the Effects (SRE) of alcohol was completed to assess the level of response to alcohol (Schuckit, Smith, & Tipp, 1997).

2.1 | Urine collection, handling, and analysis

Any turbid urine samples were centrifuged at 3,000 rpm for 15 min at room temperature. The urine was stored in three 4 ml cryovials, at a temperature of −20 °C. Urine samples from volunteers were analyzed using solid-phase extraction and ultrahigh-performance liquid chromatography (UHPLC) coupled to mass spectrometry (MS). Calibration standards were prepared by spiking blank urine with known amounts of EtG and EtS and were further pretreated as unknown samples. Ethyl-d5 sulfate and ethyl-d5 glucuronide (Medichem, Steinenbronn, BW, Germany) were added as internal standards to a 200-µl volume of sample and the solution was diluted with 800 µl acetonitrile (ACN) containing 0.1% ammonium hydroxide (NH_4OH). Oasis MAX solid-phase extraction cartridges (Waters, Milford, MA, USA) were conditioned with 1 ml methanol (MeOH) and 1 ml 0.1% NH_4OH in ACN: water 80:20% (vol/vol) before loading 1 ml of diluted sample. The cartridges were then washed with 0.5 ml ACN, and the analytes were eluted with 1 ml 0.01 M hydrochloric acid. Eluates were evaporated at 40 °C under a stream of nitrogen and reconstituted in 100 µl water. A 5-µl volume of pretreated sample was injected on a 1290 Infinity UHPLC system (Agilent Technologies, Wald-Bronn, BW, Germany) containing an Acquity UPLC HSS T3 2 mm× 100 mm column with 1.8 µm sized particles (Waters). Compounds were separated within 3 min using an eluent consisting of MeOH:25 mM formic acid 1:99% (vol/vol) at a flow rate of 0.3 ml/min and detected with a 1,100 series ion trap mass spectrometer equipped with an electrospray ionization interface (Agilent Technologies). EtG, ethyl-d5 glucuronide, EtS, and Ethyl-d5 sulfate were detected as $[M\text{-}H]^-$ ions at *m/z* 221, 226, 125, and 130, respectively.

The method was validated in urine between 0.1–40 µg/ml for EtS and 0.1–10 µg/ml for EtG.

Calibration curves were linear within this range, extraction recoveries were 86% for EtS and 93% for EtG, inter-day and intra-day accuracies were 80.7–109.8%, and inter-day and intra-day precision coefficients of variation were 3.0–17.0% (n = 6 on 3 different days, at concentration levels of 0.1, 0.2, 4.0, and 10.0 µg/ml for EtG and 0.1, 0.2, 4.0, and 40.0 µg/ml for EtS). Samples were stable for at least 12 hr at room temperature allowing overnight UHPLC–MS analysis.

In addition to EtG and EtS, urine ethanol concentrations were assessed; methodology described elsewhere (Van de Loo et al., 2016).

2.2 | Statistical analyses

Statistical analyses were performed with SPSS, version 24. Urine EtG and EtS concentration of the hangover and control day were compared using nonparametric Mann–Whitney *U* tests. In addition to EtG and EtS concentration, also their ratio (EtG/EtS) was calculated. Difference scores (post-alcohol day—control day) were calculated for each variable.

EtG and EtS concentrations were correlated with overall hangover severity, and severity scores for the individual hangover symptoms (nonparametric, Spearman's *r*), AUDIT and SRE scores, number of drinks consumed, estimated BAC, and urine ethanol concentration. The analyses were conducted for all participants together (N = 36) and separate for the hangover group and the hangover-immune group.

3 | RESULTS

Participants were aged 21 (±1.79), of which 61% men and 39% women. There were no sex differences between the hangover sensitive and resistant group. On average, participants consumed 11.6 ± 6.1 alcoholic drinks. Participants had an average score of 12.81 (±4.48) on the AUDIT and 8.35 (±1.70) on the SRE. No significant differences were found between the "hangover" and the "hangover-immune" drinkers regarding the number of alcoholic beverages consumed (12.5 vs. 10.7 drinks, p = .61), nor did their estimated peak BAC (0.19% vs. 0.17%, p = .382).

Urinary concentrations of both EtG and EtS were significantly higher on post-alcohol day compared with the control day (p = .0001). On post-alcohol day, EtG was detected in significantly higher concentrations than EtS (p = .001) in both groups.

No significant differences in EtG and EtS concentrations were observed between participants with a hangover and those claiming to be resistant to hangovers. In the hangover group, the urinary concentration of EtG was significantly increased on post-alcohol day (p = .004), as well as the urinary EtS concentration (p = .000). In the hangover-immune group, concentrations of EtG and EtS were also found to be significantly increased on post-alcohol day (p = .010 and p = .003 respectively). Urinary concentrations of EtG and EtS, and their ratio, are shown in Table 1.

For all participants (N = 36), the scores of all hangover symptoms were significantly higher on post-alcohol day, except for the symptoms 'anxiety" (p = .213) and "depression" (p = .324; for a detailed discussion, see Hogewoning et al., 2016). In the hangover group, mean (*SD*) headache severity on the post-alcohol day was 5.3 (2.9) compared to 2.0 (0.0) in the hangover-immune group. The 1-item overall hangover severity score did not significantly correlate with urinary concentrations of neither EtG, nor of EtS, nor their ratio. Regarding individual hangover symptoms, urinary EtG concentration on post-alcohol day correlated significantly only with "headache" (p = .033; r = .403). Urinary EtS concentration did not significantly correlate with any of the individual hangover symptoms. Analyzing the data separately for the hangover group and the hangover-immune group revealed that for neither of the two groups, EtG, EtS, or their ratio correlated significantly with the 1-item overall hangover severity score, or any of the individual hangover symptoms (see Table 2).

For all participants (N = 36), urine concentrations of EtG and EtS significantly correlated with urine ethanol concentrations on the post-alcohol day (p = .003, r = .533; p = .002, r = .545, respectively). No significant correlations were found with the estimated peak BAC or the number of alcoholic drinks consumed. The EtG/EtS ratio did not significantly correlate with urine ethanol concentration, estimated peak BAC, or the number of alcoholic drinks consumed.

A significant correlation between urine EtG and ethanol concentration was also found in the "hangover-immune" group, but not for

TABLE 1 Urine EtG and EtS determinations

	Subject	Control day			Post-alcohol day		
		EtG	EtS	Ratio	EtG	EtS	Ratio
Hangover group urinary EtG and EtS (μg/ml)	S101	0.00	0.00	0.00	269.630	88.220	3.06
	S102	0.00	0.00	0.00	10.620	22.860	0.46
	S103	0.030	0.245	0.12	202.550	66.550	3.04
	S104	0.00	0.262	00.00	632.725	121.500	5.21
	S105	0.00	0.083	0.00	191.370	90.000	2.13
	S106	0.131	0.00	0.00	63.060	21.750	2.90
	S107	0.127	0.063	2.00	78.050	15.720	4.97
	S108	0.405	0.758	0.53	303.650	83.225	3.65
	S109	0.058	0.059	0.99	68.870	45.280	1.52
	S110	0.00	0.00	0.00	—	60.270	—
	S111	0.015	0.102	0.15	9.866	4.204	2.35
	S112	2.226	0.337	6.60	474.025	84.150	5.63
	S113	0.015	0.046	0.33	83.250	21.710	3.83
	S114	0.052	0.017	2.98	15.350	4.910	3.13
	S115	0.138	0.162	0.85	122.980	23.910	5.14
	S116	0.379	0.753	0.50	8.000	3.500	2.29
	S117	0.00	0.00	0.00	81.200	33.760	2.41
	S118	0.015	0.039	0.39	199.640	93.230	2.14
	M	0.257	195.13	1.10	165.579	49.153	2.99
	SD	0.58	0.249	1.80	174.166	37.139	1.57
	Range	0–2.226	0–0.758	0–6.60	0–632.725	3500.00–121.500	0–5.63
Hangover-immune group urinary EtG and EtS (μg/ml)	S201	0.00	0.00	0.00	88.080	51.400	1.71
	S202	0.00	0.00	0.00	118.980	37.560	3.17
	S203	0.00	0.00	0.00	—	—	—
	S204	0.00	0.00	0.00	—	—	—
	S205	0.00	0.00	0.00	27.110	9.052	2.99
	S206	0.025	0.009	2.81	116.110	56.060	2.07
	S207	0.099	0.090	1.09	53.280	33.860	1.57
	S208	0.317	0.122	2.61	241.390	99.290	2.43
	S209	2.703	1.311	2.06	8.907	1.605	5.55
	S210	0.00	0.46	0.00	60.510	55.510	1.09
	S211	0.00	0.00	0.00	4.827	0.295	16.37
	S212	1.540	0.275	5.61	210.010	54.390	3.86
	S213	0.015	0.094	0.16	4.922	4.516	1.09
	S214	0.118	0.00	0.00	30.300	17.060	1.78
	S215	0.015	0.00	0.00	0.832	0.315	2.64
	S216	0.095	0.006	14.77	77.200	26.390	2.93
	S217	0.015	0.00	0.00	4.608	0.452	10.19
	S218	0.153	0.013	11.43	321.120	73.900	4.35
	M	0.365	0.140	2.90	85.512	32.603	3.99
	SD	0.7834	0.346	4.66	95.969	30.316	3.98
	Range	0–2.703	0–1.311	0–14.77	0–321.120	0–99.290	0–16.37

Note. Mean, *SD*, and range are presented. EtG = ethyl glucuronide; EtS = ethyl sulfate; *SD* = standard deviation.

the hangover group solely. Other correlations did not reach statistical significance (see Table 3).

4 | DISCUSSION

Urine EtG and EtS concentrations were significantly increased on post-alcohol day compared to the alcohol-free control days for all participants as well as in both groups. A significant relationship was found between their concentration and urine ethanol concentration. These findings confirm the usefulness of EtG and EtS as biomarkers for recent alcohol use. However, neither EtG, nor EtS concentrations, nor their ratio did not significantly correlated with the 1-item overall hangover severity score, nor with any of the individual hangover symptoms (with the exception of headache). Urine concentrations of EtG and EtS did not significantly differ between drinkers from the hangover group and drinkers from the hangover-immune group.

Our findings are in line with previous studies that also failed to demonstrate a significant relationship between blood EtG and EtS concentration and hangover severity (Bogstrand et al., 2014), although one study (Hoiseth et al., 2015) reported a significant relationship between EtG and EtS concentration and degree of driving impairment, (not impaired, mildly impaired, moderately impaired, or considerably impaired). However, the observed correlations were only modest (r = .170 and r = .189, respectively). Although we did not find a significant relationship between the EtG and EtS concentrations and the presence and severity of the alcohol hangover, it may be interesting for future research to also look at any relationship with performance impairment.

The only symptom that significantly correlated with EtG concentration on post-alcohol day was headache (r = .403). It is unclear why we observed this significant association with headache and not with any other hangover symptoms. Headache is commonly experienced, and was reported both on the control day (10 participants) and on the post-alcohol day (21 participants). As expected, all 15 participants that reported no headache after drinking were in the hangover-immune group. A clear distinction between the hangover group and hangover-immune group was observed regarding the severity of

TABLE 2 Correlations of urinary concentrations and ratio of EtG and EtS with hangover severity, hangover symptoms, and urinary ethanol concentrations

	Hangover group			Hangover-immune group		
	EtG	EtS	EtG/EtS ratio	EtG	EtS	EtG/EtS ratio
1-item overall hangover score	0.120	0.194	−0.098	−0.425	−0.314	−0.092
Sleepiness	0.322	0.463	−0.169	−0.284	−0.119	0.137
Sweating	−0.176	−0.229	0.064	0.328	0.281	0.328
Concentration problems	0.233	0.458	−0.449	−0.145	−0.116	0.145
Nausea	0.216	0.286	−0.069	0.000	0.152	0.152
Thirst	−0.408	−0.174	−0.453	0.125	0.116	0.018
Sleep problems	−0.032	−0.040	0.296	−0.378	−0.241	0.310
Heart racing	0.372	0.062	0.554	−0.034	−s0.103	0.310
Dizziness	0.058	0.078	−0.047	0.034	0.103	−0.310
Confusion	0.218	0.098	0.145	0.378	0.447	−0.103
Shaking	0.147	0.152	0.680	0.034	0.103	−0.310
Headache	0.155	0.264	−0.093	0.199	0.126	0.297
Regret	0.177	0.298	−0.170	0.234	0.094	0.469
Weakness	−0.257	−0.117	−0.182	−0.090	0.036	0.049
Clumsy	0.069	0.149	−0.141	−0.436	−0.240	−0.157
Stomach pain[a]	0.022	−0.057	0.262	—	—	—
Heart beating[a]	0.307	0.298	0.165	—	—	—
Anxiety	0.046	−0.220	0.407	−0.034	−0.103	0.310
Depression	0.141	−0.085	0.328	−0.034	−0.103	0.310
Reduced appetite	0.313	0.386	0.005	0.051	0.101	0.405
Light sensitivity	−0.118	0.062	−0.234	0.447	0.182	0.241
Vomiting	0.329	0.313	0.095	−0.103	−0.241	0.447
Tired	−0.069	0.184	−0.508	−0.216	0.009	−0.171
Apathy	0.154	0.229	−0.013	0.094	0.133	0.400

[a]Difference scores for heart beating and stomach pain were zero for each individual in the hangover-immune group.

TABLE 3 Correlations of urinary EtG and EtS concentrations, and EtG to EtS ratio with urinary ethanol concentrations, estimated BAC, and number of alcoholic drinks

	Overall			Hangover group			Hangover-immune group		
	EtG	EtS	Ratio	EtG	EtS	Ratio	EtG	EtS	Ratio
Urinary ethanol (mg/ml)	0.533**	0.545**	−0.032	0.433	0.400	−0.042	0.660*	0.513	0.304
eBAC	0.219	0.128	0.132	0.007	−0.100	0.103	0.165	0.533	0.108
Number of alcoholic drinks	0.267	0.225	−0.077*	0.133	0.054	0.049	0.523	0.483	0.046

Note. Correlations are shown for the overall population, and for the "hangover" and "hangover-immune" group separately. BAC = blood alcohol concentrations; EtG = ethyl glucuronide; EtS = ethyl sulfate.

*$p < .05$.

**$p < .01$.

headache, finding higher scores on headache in the hangover group compared to the hangover-immune group.

As for any study using a naturalistic design, some common limitations also apply for this study. For instance, factors such as alcohol consumption and participant behavior were not controlled. This means that activities of participants varied (e.g., dancing or drinking in a pub), as did the type of alcoholic beverage consumed. Although urine samples were collected at a fixed time point for every participant, the time between the last alcoholic consumption and urine collection also varied between participants. Although beverage consumption during the drinking session was recorded, no information was collected on urine voiding during drinking or during the night. The possible consumption of water during the night or early morning may have diluted the concentration of EtG and EtS in the bladder. Voiding during night or in the early morning may have led to the excretion of EtG and EtS. There is however no reason to assume that these behaviors have differed between the hangover group and hangover-immune group, as these did not differ significantly on total alcohol consumed and estimated BAC.

In summary, urinary concentrations of EtG and EtS are unrelated to the presence and severity of the alcohol hangover. Nevertheless,

EtG and EtS may be useful markers of recent alcohol consumption, as both significantly correlate with urinary ethanol concentrations on post-alcohol day.

CONFLICT OF INTERESTS

Joris Verster has received grants/research support from the Dutch Ministry of Infrastructure and the Environment, Janssen, Nutricia, Red Bull, and Takeda, and has acted as a consultant for the Canadian Beverage Association, Centraal Bureau Drogisterijbedrijven, Coleman Frost, Danone, Deenox, Eisai, Janssen, Jazz, Purdue, Red Bull, Sanofi-Aventis, Sen-Jam Pharmaceutical, Sepracor, Takeda, Transcept, Trimbos Institute, and Vital Beverages. Karel Brookhuis has received grants/research support from NWO, the Dutch Ministry of Infrastructure and the Environment, European Commission, Wyeth, Sanofi, Schering, Nissan, JARI, Mercedes Benz, and Verbond van Verzekeraars. Aletta Kraneveld has received grants/research support from Top Institue Pharma, NOW, Janssen, GSK, Nutricia Research, and Friesland Campina. Johan Garssen is part-time employee of Nutricia Research and received research grants from Nutricia research foundation, Top Institute Pharma, Top Institute Food and Nutrition, GSK, STW, NWO, Friesland Campina, CCC, Raak-Pro, and EU. The other authors have no potential conflicts of interest to disclose.

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