Longitudinal study of the breath of healthy volunteers using SIFT-MS

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Monitoring breath in healthy volunteers

- Finding out what is normal
- Extending studies already done
- Greater number of volunteers
- Longer duration of study

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Experimental plan

- 30 volunteers recruited, 11 F 19 M
- range of ages (24-59)
- range of BMIs (18.4 to 30.1)
- 3 smokers, 27 non-smokers
- weekly morning samples given for 6 months
- questionnaires completed for each sample
- 6 sets of breath samples taken each time to analyse main metabolites and other compounds

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Questionnaire details

- Age, gender
- Weight, height (body mass index, BMI)
- Health and stressed or calm
- Diet over 24 hours
- Alcohol consumed over 24 hours
- Cigarettes smoked
- Artificial sweetener consumption
- Medication taken
- How well volunteer slept
- Exercise taken

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Selected ion flow tube mass spectrometry (SIFT-MS)….1

- trace gas analyser
- uses one of 3 selected precursor ions; rapid switching between precursors
- precursors selected with upstream quadrupole
- precursors react with trace gas species but not air components
- exploits chemical ionisation of species

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Selected ion flow tube mass spectrometry (SIFT-MS)….2

- downstream quadrupole separates product ions
- quantification possible with knowledge of reaction kinetics
- can operate in quantitative mode (MUI) for looking at specific ions
- can operate in semi quantitative mode (MSE) to do whole mass spectrum

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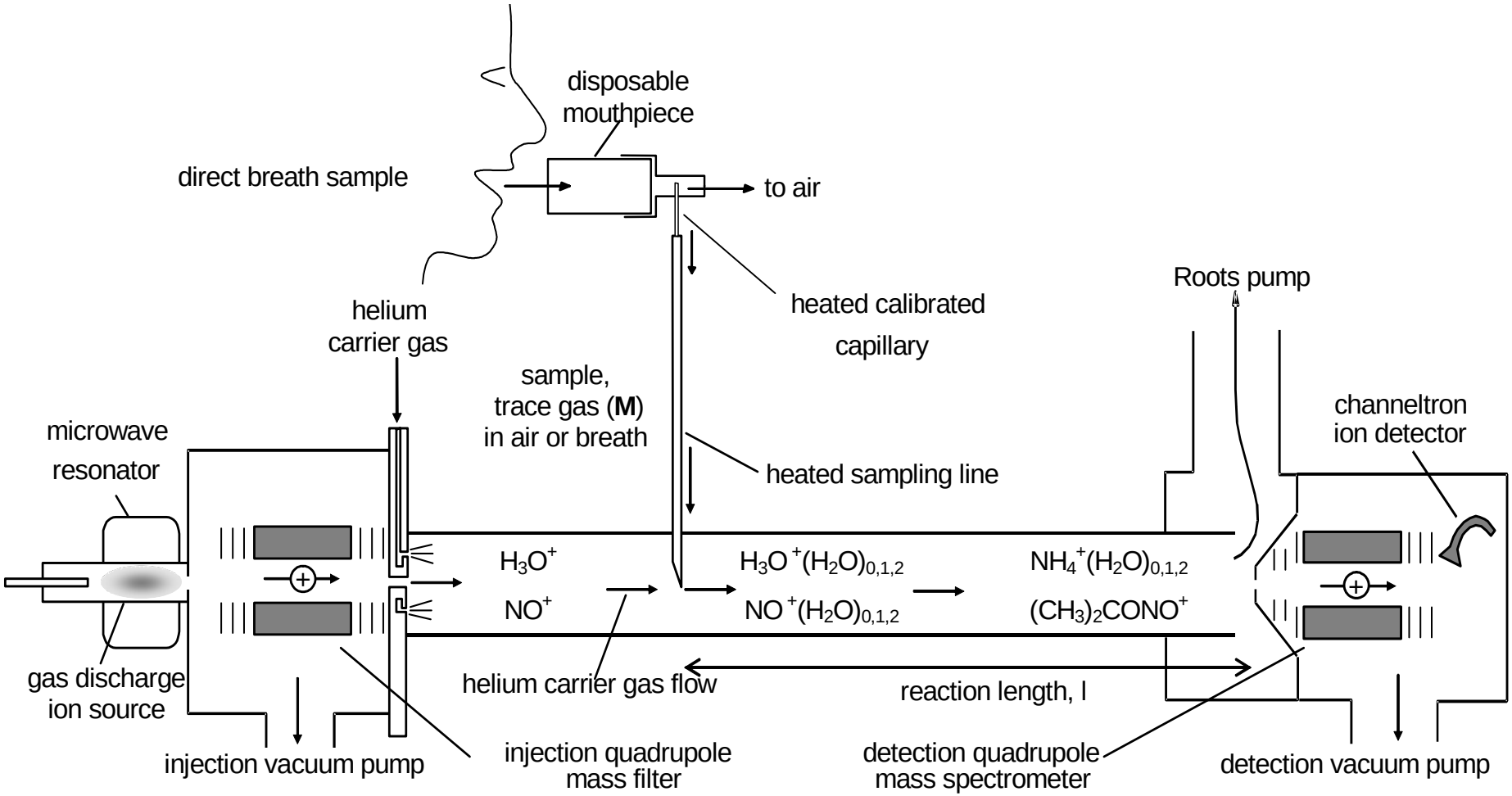
disposable
mouthpiece
direct breath sample
to air
helium
carrier gas
heated calibrated
capillary
Roots pump
sample,
trace gas (M)
in air or breath
channeltron
ion detector
microwave
resonator
heated sampling line
H_3O^+
NO^+
$H_3O^+(H_2O)_{0,1,2}$
$NO^+(H_2O)_{0,1,2}$
$NH_4^+(H_2O)_{0,1,2}$
$(CH_3)_2CONO^+$
gas discharge
ion source
helium carrier gas flow
reaction length, l
injection vacuum pump
injection quadrupole
mass filter
detection quadrupole
mass spectrometer
detection vacuum pump

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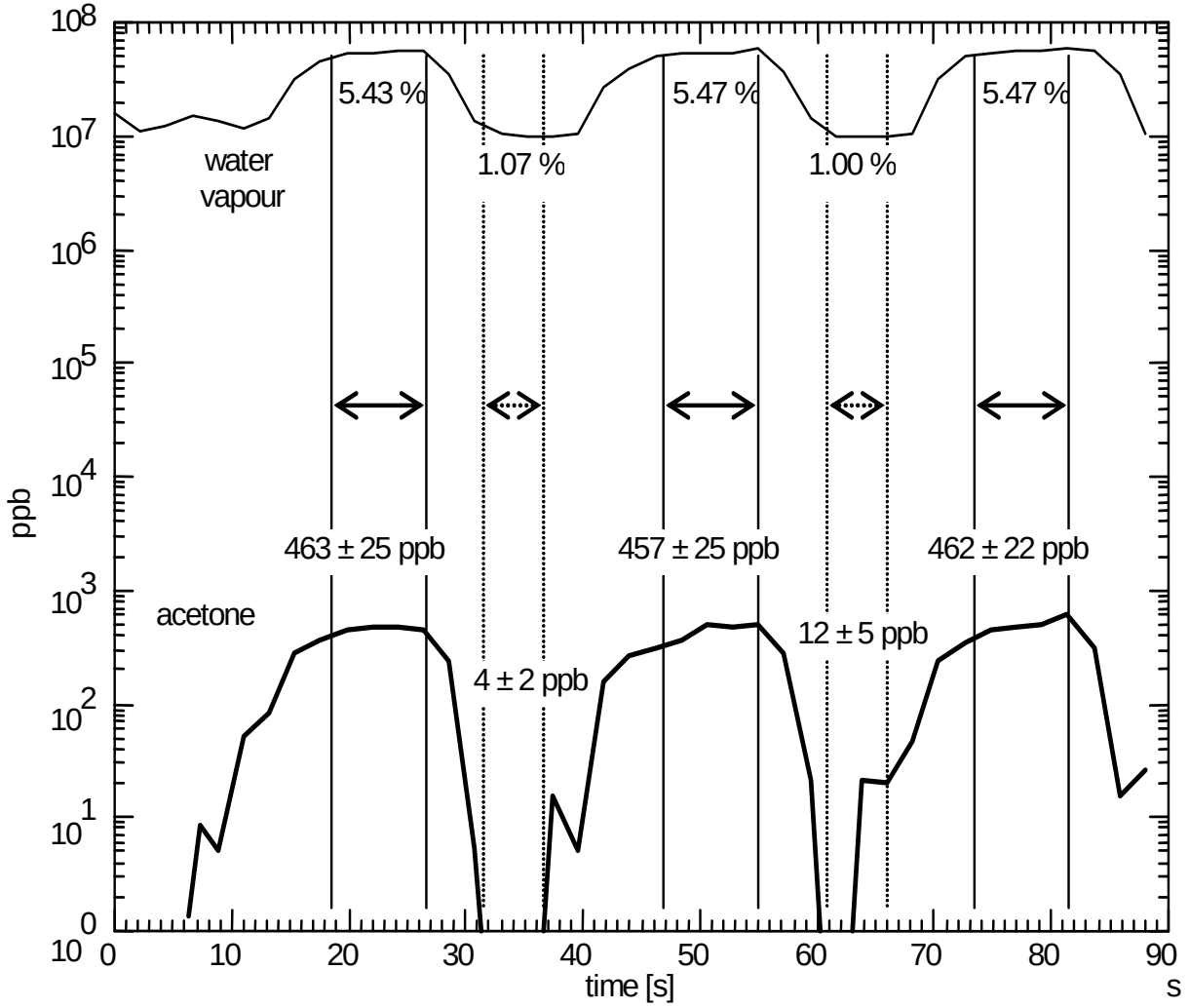
water vapour
5.43 %
1.07 %
5.47 %
1.00 %
5.47 %
463 ± 25 ppb
457 ± 25 ppb
462 ± 22 ppb
acetone
4 ± 2 ppb
12 ± 5 ppb
ppb
time [s]
s

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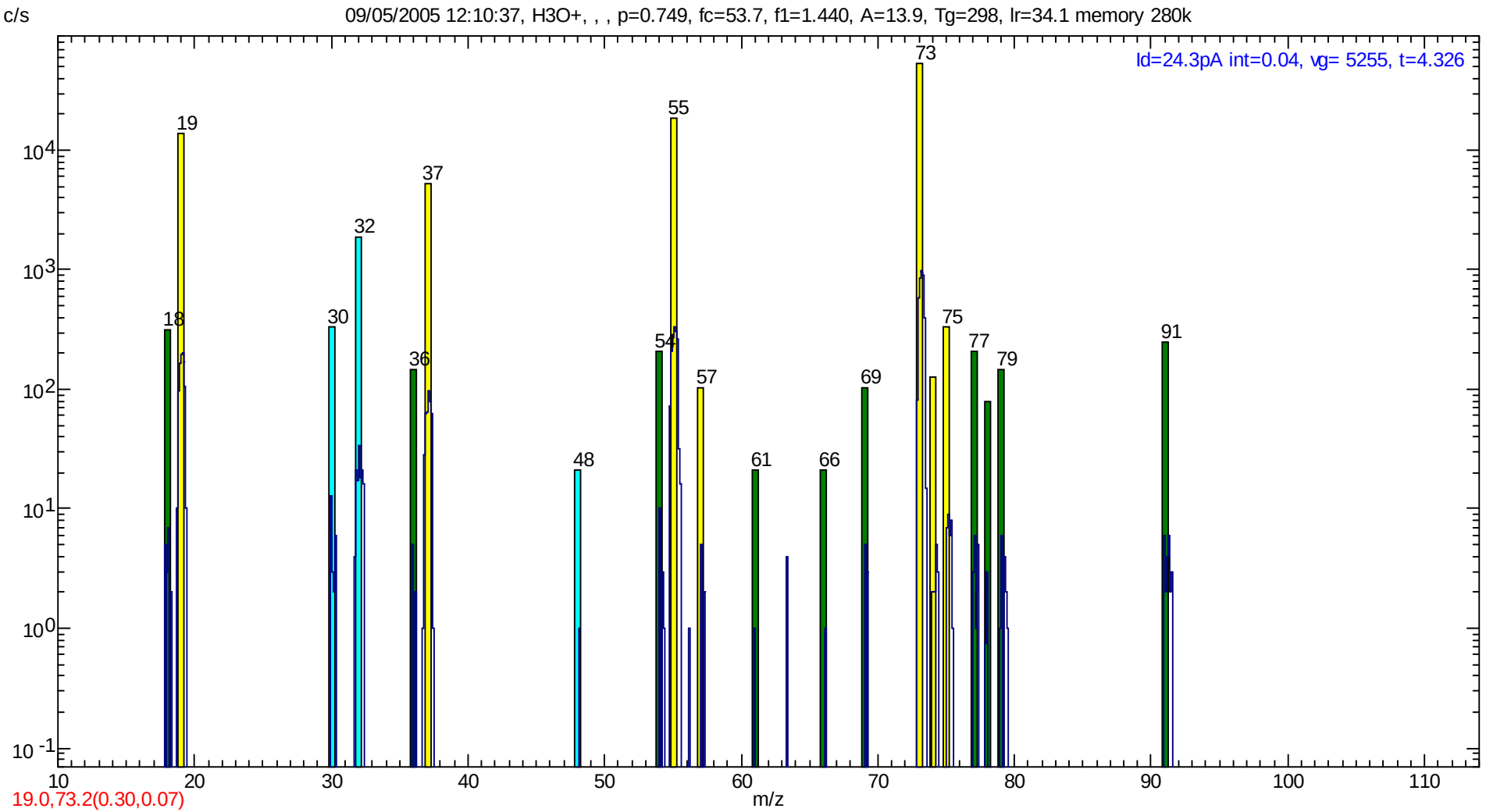

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Advantages of SIFT-MS

- direct or off-line samples
- real time analysis (typically just a few seconds)
- wide dynamic range of different compounds in same sample (low ppb to %)
- absolute quantification (unlike GC-MS)
- water saturated samples cause no problems
- can rapidly use all precursor ions for identification of sample

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SIFT MS analysis

- use of three precursor ions
- for each breath volunteer's weekly visit:
  - quantitative MUI profiles taken of main breath components
  - less quantitative MSE scans taken using each precursor to identify other breath compounds

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Major volatile breath metabolites

- isoprene – *cholesterol, organ failure*
- ethanol – *gut flora activity*
- acetaldehyde – *tumour marker?*
- ammonia – *elevated in uraemia, liver disease*
- acetone – *elevated in diabetes*
- methanol *?*
- (iso) – propanol - *diabetes?*
- acetonitrile - *smoking*

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Isoprene results

- 481 samples taken
- range: 0 to 474 ppb
- mean value: 118 ppb, s.d. 68 ppb
- no correlation observed with cholesterol levels
- moderate exercise rapidly increases breath isoprene
- no correlation with age, gender or bmi

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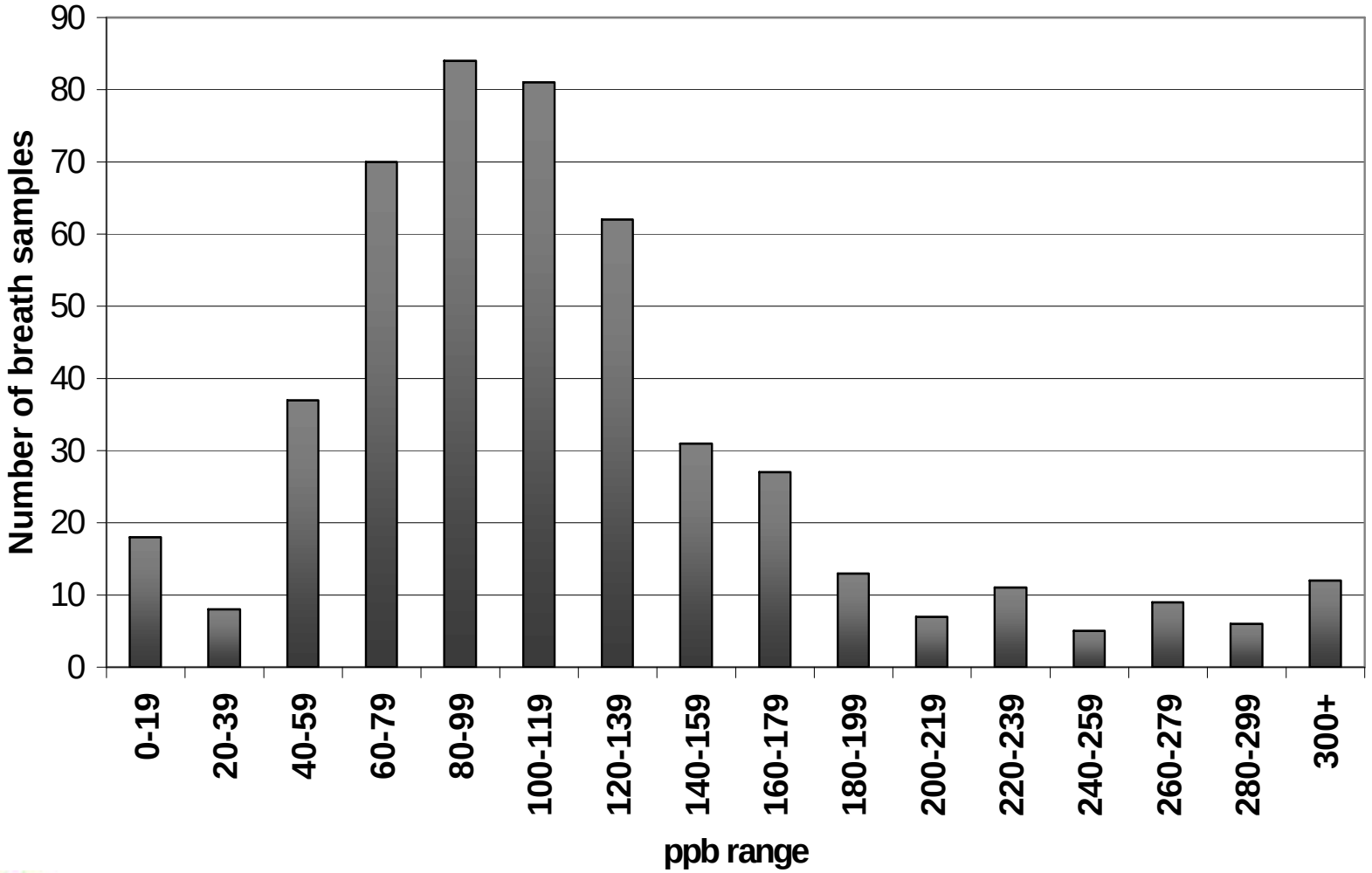
Isoprene distribution



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Ethanol and acetaldehyde

- 478 ethanol tests
  - range 0 - 1663 ppb,
  - mean 196 ppb
  - s.d. 244 ppb
- 391 acetaldehyde tests
  - range 0 to 104 ppb
  - mean 24 ppb
  - s.d. 17 ppb

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Ethanol and acetaldehyde (2)

- sugar consumed within 2 hours of sample affects breath ethanol
- acetaldehyde is unaffected by sugar
- ethanol and acetaldehyde are not correlated
- ethanol is not correlated with alcohol consumed more than 9 hours prior to test
- both endogenous
- some background ethanol; no acetaldehyde

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Ethanol and acetaldehyde distributions

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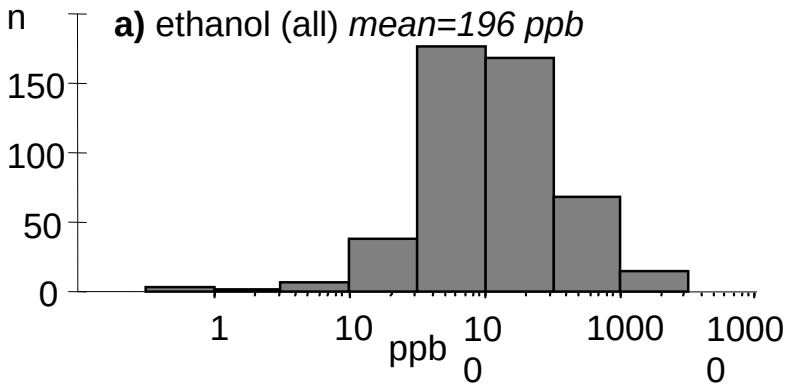

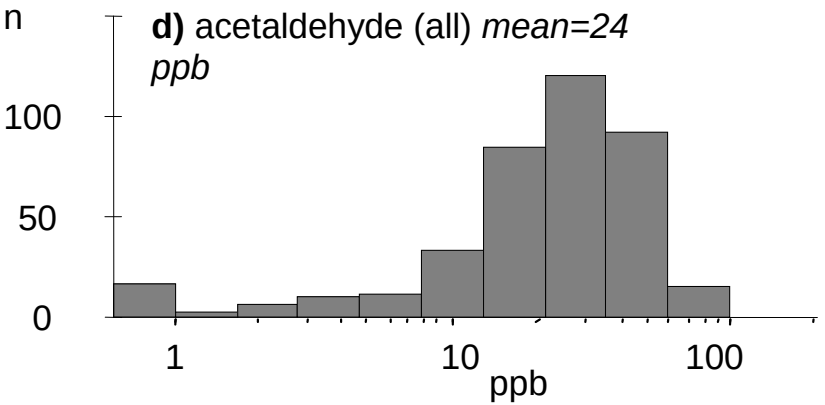

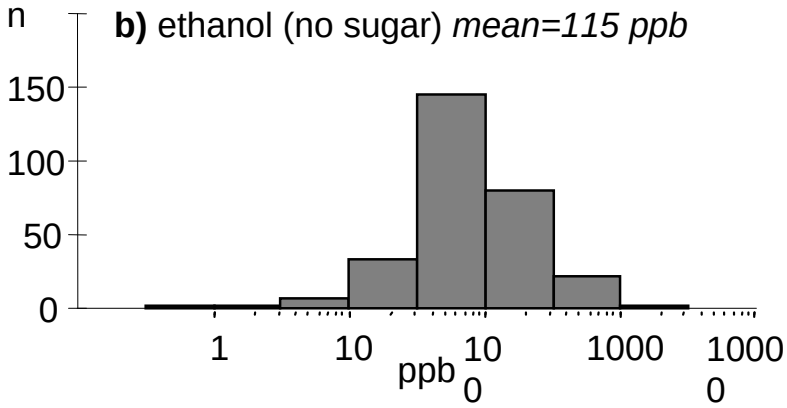

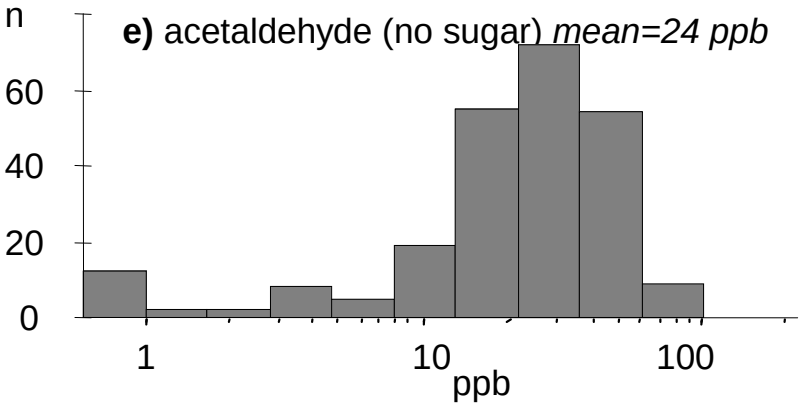

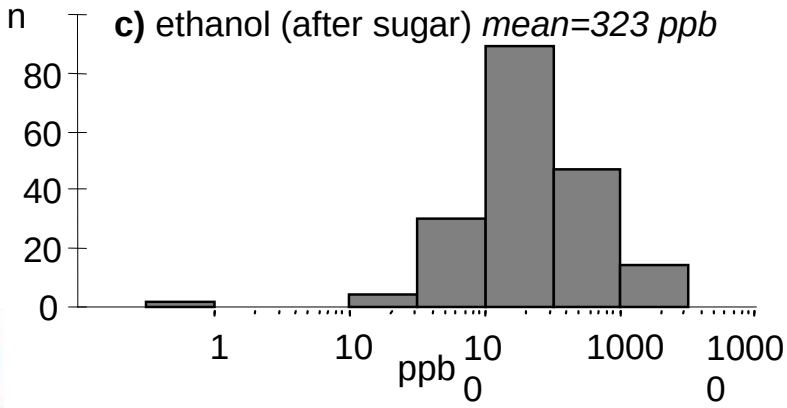

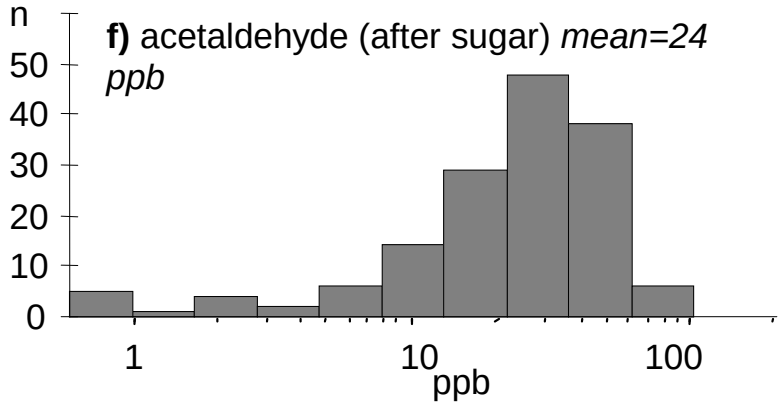

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Ammonia

- 480 samples taken
- range: 248 to 2935 ppb
- mean value: 933 ppb, s.d. 461 ppb
- strong correlation observed with age
- no correlation observed with gender or diet
- may be influenced by background levels

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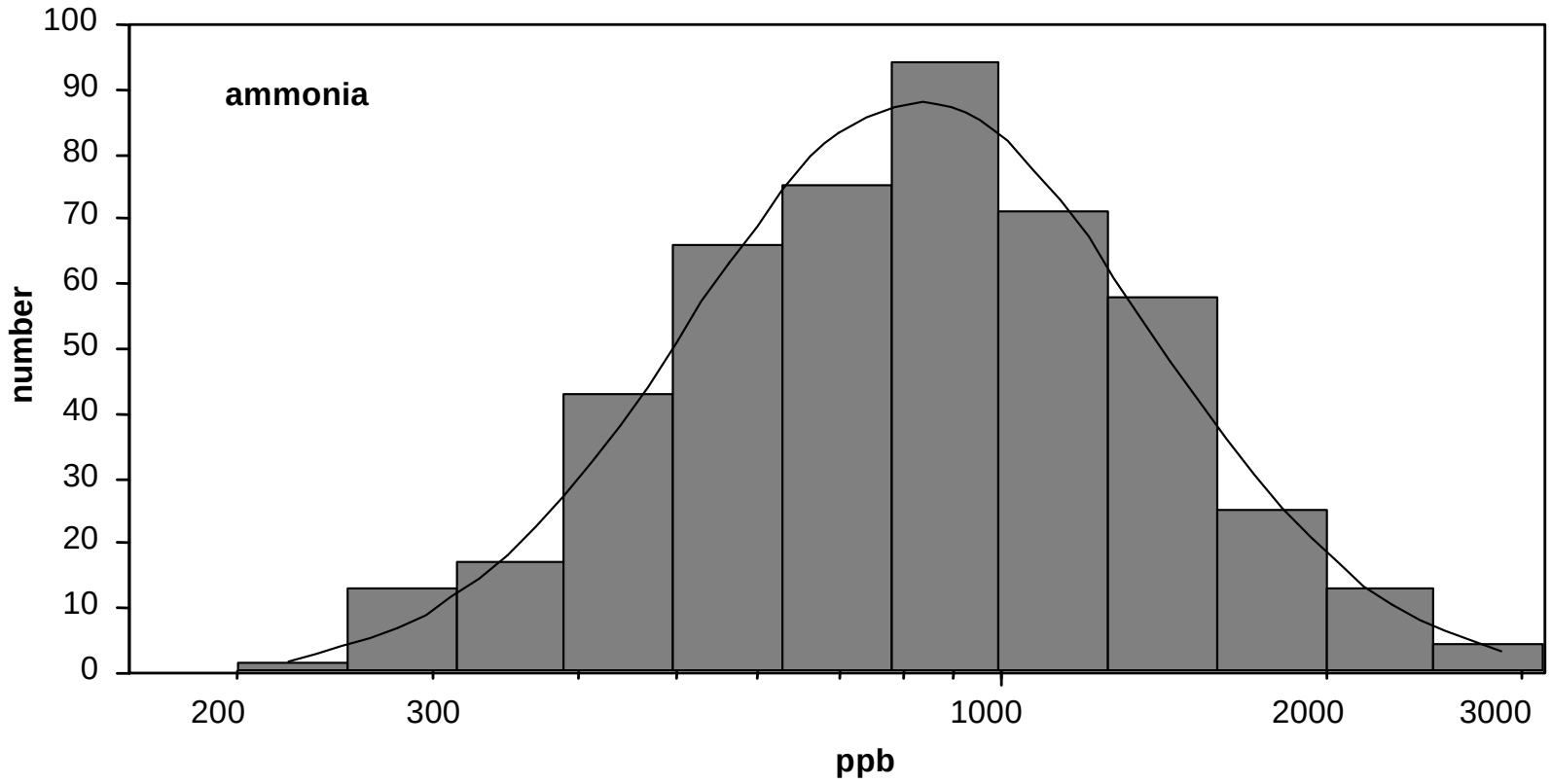
Ammonia distribution



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Acetone

- 481 samples taken
- range: 148 to 2744 ppb
- mean value: 535 ppb, s.d. 297 ppb
- acetone is correlated with calories consumed
- no strong correlation with bmi
- difference in acetone levels between men and women (men > women)

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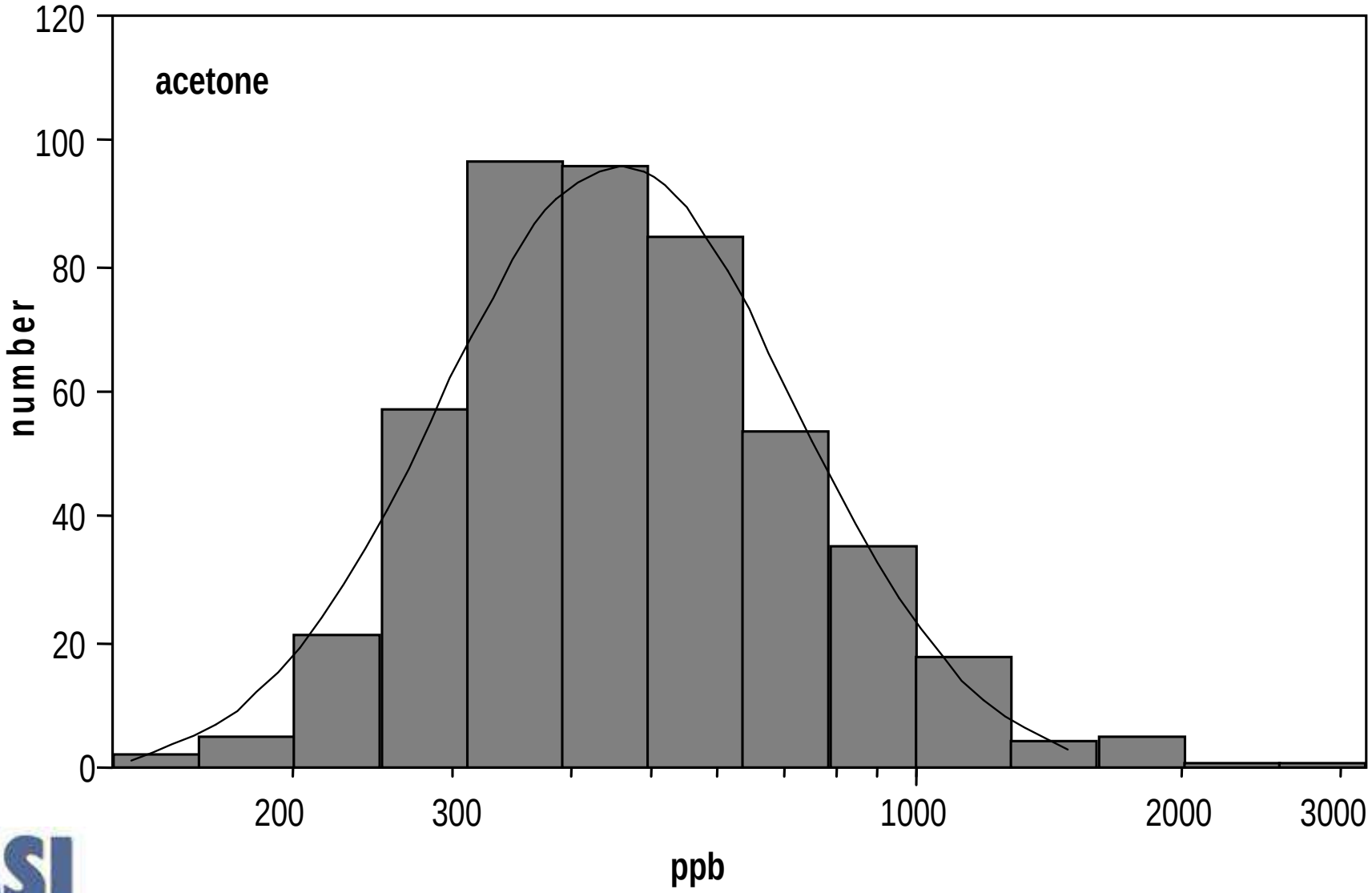
Acetone distribution



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(Iso) - propanol

- mean value 22 ppb, s.d. 17 ppb
- weak but significant correlation between (iso)propanol and acetone
- possibly significant in diabetes

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Methanol

- 477 samples taken
- range: 32 to 1684 ppb
- mean value: 502 ppb, s.d. 239 ppb
- methanol decreases with increasing BMI
- slight increase in methanol after fruit consumption
- no correlation of methanol with breath ethanol or ethanol units consumed in previous 24 hours

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Summary – compounds and means

Isoprene	118
Ethanol	196
Acetaldehyde	24
Ammonia	933
Acetone	535
(Iso) propanol	22
Methanol	502

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Implications

- extending limits of normal ranges
- isoprene range a surprise
- “contamination” by mouth flora sometimes significant; perhaps gut flora
- background levels *may* be significant, especially with ammonia

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Future work

- multivariate statistical analysis of all data
- unique “fingerprints” may be possible
- examining “mse” scan data for additional breath compounds present - identification
- using data to produce “healthy profile” - extend to disease diagnosis
- looking at breath compounds in disease states
- new, small instrument now available

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