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A human exposure study to investigate biological monitoring methods for 2-butoxyethanol

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2-Butoxyethanol is a glycol ether widely used in printing inks, varnishes and cleaning fluids. As skin absorption can be significant, biological monitoring is useful in monitoring worker exposure. A number of analytes and matrices have been used previously, including 2-butoxyethanol in blood and free and total 2-butoxyacetic acid in urine. Using a combination of a volunteer study and samples from exposed workers, we compared the applicability of some of the biological monitoring markers available. We conclude that 2-butoxyethanol in blood is not a suitable marker for biological monitoring due to sampling problems. In view of the low-level exposures reported in occupational surveys, 2-butoxyethanol in breath is also unsuitable because of a lack of sensitivity. Measuring 2-butoxyacetic acid in blood is possible, although non-invasive urine samples are preferred. Free 2-butoxyacetic acid in urine has previously been widely used; however, we found that the extent of conjugation of 2-butoxyacetic acid in urine varied from 0 to 100% both within and between individuals and is not related to time, concentration or urine pH. Data from 48 exposed workers suggested that an estimated 57% (95% confidence interval 44–70%) of the total 2-butoxyacetic acid is excreted in the conjugated form, and that conjugation may be activated above a certain exposure level. Using total 2-butoxyacetic acid significantly reduced inter-individual variation. Elimination half-lives for free and total 2-butoxyacetic acid were similar ($\sim$6 h) and there was no delay in excretion of the conjugated metabolite (peak excretion for both free and total was between 6 and 12 h after the end of exposure). In conclusion, we propose that total butoxyacetic acid (after acid hydrolysis) in urine is the biomarker of choice for monitoring exposure to 2-butoxyethanol. Urine samples should be collected post-shift towards the end of the working week.

Keywords: biological monitoring, urine, butoxyethanol, conjugation.

Introduction

2-Butoxyethanol is a glycol ether widely used in printing inks, varnishes and cleaning fluids. Its miscibility with both water and a large number of organic solvents makes it particularly useful as a solvent in oil–water compositions. Unlike other small chain ethylene glycol ethers such as 2-methoxyethanol and 2-ethoxyethanol, 2-butoxyethanol has not been shown to be reprotoxic; rather its main toxicological effect is haemolytic anaemia (Ghanayem and Sullivan 1993). This lower toxicity has resulted in 2-butoxyethanol being the most widely used glycol ether worldwide. The industrial uses of 2-butoxyethanol, particularly in cleaning fluids, mean that dermal absorption is a likely source of exposure, and regulatory bodies have given it a 'skin' notation (American Conference of Industrial Hygienists (ACGIH) 2003, Deutsche Forschungsgemeinshaft (DFG) 2002, Health and Safety Executive (HSE) 2002). Given the widespread use of 2-

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butoxyethanol and the scope for dermal contact and absorption, biological monitoring is recommended for a complete exposure assessment.

A number of biological markers have been used to determine the body burden after exposure to 2-butoxyethanol, including 2-butoxyethanol in blood (Johanson and Boman 1991, Corley *et al*. 1997), 2-butoxyacetic acid in blood (Corley *et al.* 1997) and 2-butoxyacetic acid in urine, both free (Johanson *et al.* 1986, Angerer *et al.* 1990, Vincent *et al.* 1993, Laitinen 1998) and total (Rettenmeier *et al.* 1993, Sakai *et al.* 1994, Corley *et al.* 1997). Inhalation exposures were generally low in the field studies (less than 2 p.p.m. with a maximum exposure of 8 p.p.m.). A controlled human volunteer study with higher levels of exposure was reported by Johanson *et al.* (1986), but only free 2-butoxyacetic acid levels in urine were measured.

Work by Rettenmeier *et al.* (1993) and supported by Sakai *et al.* (1994) and Corley *et al.* (1997) showed that 2-butoxyacetic acid excreted in urine may be conjugated to glutamine and that the extent of conjugation may vary markedly. Rettenmeier *et al.* (1993) suggested that conjugation might become saturated at higher doses.

Because biological monitoring is a potentially useful means of assessing exposure and the efficacy of control measures for 2-butoxyethanol, some countries have already established guidance values. In the UK the biological monitoring guidance value for 2-butoxyacetic acid in urine is 240 mmol mol^{-1} creatinine. The Deutsche Forschungsgemeinschaft (DFG) has set a biological tolerance (BAT) value of 100 mg l^{-1} for 2-butoxyacetic acid in urine. Both of these guidance values are based on free 2-butoxyacetic acid and both recommend sampling post-shift towards the end of the working week. The DFG stated that measuring the conjugate in addition to the free 2-butoxyacetic acid would be sensible. However, in view of the limited data on conjugation of 2-butoxyacetic acid at that time (1994), the bias in occupational studies towards free 2-butoxyacetic acid, their opinion that conjugation lessened with increased exposure and the fact that free 2-butoxyacetic acid is the toxic metabolite, the BAT was determined as the level of free 2-butoxyacetic acid in urine.

This paper presents a human volunteer study looking at whole body exposure to 2-butoxyethanol vapour via all routes (inhalation, ingestion and dermal) and by skin only. A range of potential biological markers were also evaluated to propose a biological monitoring strategy for 2-butoxyethanol. In addition, results from occupationally exposed workers are presented.

Methods

Four volunteers (two males and two females, aged 28–33 years) took part in the study, which was approved by the Health and Safety Executive's Research Ethics Committee. The subjects were exposed (whole body) on two separate occasions to 50 p.p.m. 2-butoxyethanol for 2 h. On one occasion the volunteers were exposed both by inhalation and dermally to 2-butoxyethanol. On the second occasion, volunteers wore air-fed half-masks so that they could breathe clean air whilst being exposed dermally to 2-butoxyethanol vapour. Exposures were performed in the Health and Safety Laboratory Controlled Atmosphere Facility (CAF), a purpose-built room approximately 8 m^3 in volume. The methodology has been previously described by Brooke *et al.* (1998).

In addition, results are reported from 48 post-shift urine samples collected from workers occupationally exposed to 2-butoxyethanol during silk-screen printing and analysed for free and total 2-butoxyacetic acid.

Biological monitoring

For the two volunteer studies, a cannula was inserted into a forearm vein and blood was collected before, during and after each exposure (at 0, 0.5, 1, 1.5 and 2 h, and every 20 min post-exposure for a further 2 h). Prior to venepuncture, the skin surface was cleaned with isopropyl alcohol, which did not interfere with subsequent analytical procedures. Blood samples were stored for a maximum of 48 h as whole blood at 4°C until analysed.

Volunteers provided breath samples before and immediately after exposure (at 0 and 2 h) and then at 10–15 min intervals for a further 2 h. End-tidal breath samples were taken using a Bio-VOC™ breath sampler (Markes International, Llantisart, Wales) into tubes containing Tenax, and analysed by thermal desorption with gas chromatography-mass spectroscopy (GC-MS) as previously reported (Dyne *et al.* 1997). For the study involving the wearing of air-fed masks, two breath samples were combined to improve detection. For the immediate post-exposure breath sample following skin-only exposure, volunteers took a final breath of solvent-free air via the mask and were asked to hold their breath prior to leaving the chamber and providing a sample, in order to reduce the potential for inhalation uptake from contaminated clothing. Clothing was then changed to ensure that further uptake was prevented.

Volunteers provided urine samples before and after each exposure (at 0, 4, 6, 8, 10, 12, 22, 26, 30 and 34 h). The urine volume was recorded and samples were stored at −20°C until analysed. All blood and urine samples were analysed in duplicate. Creatinine concentrations were measured for urine samples according to the method of Jaffe (1886), and urine concentrations were reported as millimoles of 2-butoxyacetic acid per mole of creatinine.

2-Butoxyethanol

Blood samples were analysed for 2-butoxyethanol. Sample aliquots (200 µl), the internal standard (heptanol 200 µl) and a concentrated salt solution (K_2HPO_4 200 µl) were mixed and extracted into toluene (2 ml). The organic layer was transferred, and pyridine (20 µl) and pentafluorobenzoyl chloride (10 µl) were added. Samples were derivatized at 50°C for 1 h. The reagents were then evaporated and the residue reconstituted in 90% methanol (1 ml) and extracted into hexane (2 ml). The hexane layer was washed with 90% methanol (1 ml), and an aliquot (1 µl) of the hexane layer was then injected splitless (30 s) into a gas chromatograph with electron capture detection. The oven was held at 150°C, the injector at 220°C, and the detector at 200°C. A BP5 fused silica capillary column (50 m × 0.33 mm internal diameter, 0.25 µm film) was used.

2-Butoxyacetic acid

Blood and urine were analysed for both free and total 2-butoxyacetic acid using the following procedure. An aliquot of sample (50 µl) was added to 1 ml acetone. The internal standard (propoxyacetic acid), anhydrous potassium carbonate and pentafluorobenzyl bromide (50 µl) were added. Samples were then mixed and derivatized at 90°C for 1 h in sealed tubes. After cooling, an aliquot was transferred to gas chromatograph vials prior to analysis. For the analysis of total 2-butoxyacetic acid, the sample was hydrolysed with concentrated hydrochloric acid (50 µl) at 90°C for 1 h in sealed tubes before being added to the acetone and treated as above. Analysis was performed by GC-MS (HP 5973, Agilent, South Queensferry, UK) with negative ion chemical ionization, using methane as the reagent gas. Aliquots (1 µl) were injected splitless (30 s) at 350°C into a ZB-1 column (Phenomenex, Macclesfield, UK) (30 m × 0.32 mm internal diameter, 1 µm film). The oven temperature was initially 100°C (held for 1 min) and was then increased at 10°C/min to 200°C, then increased at 20°C/min to 220°C, where it was held for 1 min. The fragment ions monitored had a mass-to charge ratio (m/z) of 117 for propoxyacetic acid (the internal standard) and 131 for 2-butoxyacetic acid. The source temperature was 150°C and the quadrupole temperature was 100°C.

Calculation of the absorbed dose

Breath values are expressed as aggregated measurements over the post-exposure collection period. It was assumed that post-exposure breathing rates were consistent for an individual in both parts of the study, and that elimination via the respiratory tract was unaffected by the type of exposure received. Urinary elimination is expressed as the cumulative total analyte excreted over the post-exposure collection period, calculated by summing the products of the analyte concentration and urine volume for the individual time points. Estimates based on blood sampling were calculated simply as the sum of the 2-butoxyethanol concentration at each time point, as the volume of circulating blood was assumed to be constant for the two studies. For each medium analysed, the results obtained for skin-only exposure are

Table 1. Percentage dermal absorption contribution to the total body burden as estimated from various analytes and biological media ($n = 4$).

Biological medium	Analyte	Mean dermal absorption (%)	Range (%)
Blood	2-Butoxyethanol	124	104–139
	2-Butoxyacetic acid	12	5–17
Breath	2-Butoxyethanol	6	5–8
Urine (unhydrolysed)	'Free' 2-butoxyacetic acid	23	10–31
Urine (hydrolysed)	'Total' 2-butoxyacetic acid	11	9–14

expressed as a percentage of the 'whole body' exposure (inhalation, ingestion and dermal exposure) in order to obtain an estimate of the uptake via the dermal route, with each volunteer acting as their own control. In making these comparisons it is assumed that, following uptake into the body, the distribution, metabolism and excretion of these substances is identical under both sets of exposure conditions.

Results and discussion

The percentage dermal contribution to total body burden as estimated from various analytes and sample media are given in Table 1.

2-Butoxyethanol in blood

As can be seen from Table 1, using 2-butoxyethanol in blood gives an estimated dermal absorption of $> 100\%$. Although venous rather than capillary samples were taken, this finding may support the theory of Corley *et al.* (1994) that blood sampled from an exposed skin site may have high localized concentrations of 2-butoxyethanol. This data may also explain why Johanson and Boman (1991) obtained such a high estimate of the dermal contribution to the total body burden. However, as shown in Table 1, the high estimate obtained from using blood 2-butoxyethanol levels does not agree with any of the other analytes or sampling media. Our data showed an average peak blood concentration of 7 μM (50 p.p.m. for 2 h at rest). Johanson and Boman (1991) reported a peak of 7.4 μM for a 20 p.p.m. exposure for 2 h at 50 W work; although the inhalation concentration used was lower, the increased respiration due to exercise make the results roughly comparable. Our data gave a half-life of 56 min (range 41–84 min) for 2-butoxyethanol in blood, which again is comparable with that of Johanson and Boman (1991) (40 min, range 21–63 min) and Corley *et al.* (1997) (36–43 min). Corley *et al.* (1997) conducted a study involving single-arm exposure to 50 p.p.m. 2-butoxyethanol for 2 h. Post-exposure blood levels in the exposed arm reached an average of 36 μM (range 20–86 μM), whereas levels in the unexposed arm only reached 0.037 μM. Corley *et al.* (1997) explained this large difference in blood levels as being due to high local concentrations in the exposed arm from venous blood draining the skin. The peak blood values for the study reported here and for Johanson and Boman (1991) are five times less than those in the study of Corley *et al*. (1997). Our study does, however, support the view of Corley *et al.* (1997) that 2-butoxyethanol blood measurements are unsuitable for biological monitoring of 2-butoxyethanol exposure, particularly if there is dermal exposure.

2-Butoxyacetic acid in blood

Determination of 2-butoxyacetic acid in blood did not give rise to the apparently excessive dermal absorption estimate produced by measuring 2-butoxyethanol in blood. This is in agreement with the data from Corley *et al.* (1997). Peak levels of 2-butoxyacetic acid were seen 20 min post-exposure, with an average concentration of 35 μM (range 28–43 μM) and a half-life in blood of 13 min (range 2–42 min). Johanson and Johnsson (1991) reported blood 2-butoxyacetic acid levels of 22–60 μM (after 2 h' exposure to 20 p.p.m. at 50 W work) and a half-life of 4 h. The difference in half-lives between our results and those of Johanson and Johnsson (1991) can be in part explained by the different sampling strategies. Our study took post-exposure samples every 20 min for 2 h (and thus looked at initial elimination), whereas the study of Johanson and Johnsson (1991) took samples 2, 4 and 6 h after exposure (and thus looked at a second, longer half-life). Our blood 2-butoxyacetic acid data give a dermal absorption estimate of 12%, which is in good agreement with the physiologically based pharmacokinetic prediction of 15% under similar conditions made by Corley *et al*. (1997).

2-Butoxyethanol in breath

Measuring 2-butoxyethanol in breath gave the lowest estimate of the dermal absorption contribution to the total body burden. This may in part be due to the lack of sensitivity of the technique with this analyte and the need to take combined samples (to gain sufficient sensitivity) in the low-exposure dermal study. The highest breath value in the dermal-only study was only 12 times the detection limit. The lack of sensitivity of 2-butoxyethanol in breath may be due to a combination of low volatility and high water solubility, reducing its concentration in breath. Although suitable for monitoring 2-butoxyethanol exposure around the occupational exposure limit, the poor sensitivity of the 2-butoxyethanol in breath method restricts its ability to monitor the low levels of exposure typically found in field studies.

2-Butoxyacetic acid in urine

Using free 2-butoxyacetic acid in urine gives an estimated dermal absorption of 23%. However, there is a large variation in results between volunteers (range 10–31%, coefficient of variation [CV] 66%). By contrast, the use of total 2-butoxyacetic acid gives a lower estimate of dermal absorption (11%), but there is better agreement between volunteers (range 9–14%, CV 23%). The mean excretion curves for free and total 2-butoxyacetic acid in urine are presented in Figure 1.

Figure 1 shows that the pattern of excretion and the elimination half-life are similar for both free (mean 5.9 ± 1.9 h) and total (mean 6.1 ± 2.4 h) 2-butoxyacetic acid, indicating that conjugation does not delay elimination. It is interesting to note that even for inhalation exposure, the peak excretion occurs some 6–12 h after the end of exposure. This is comparable to the results of Johanson *et al.* (1986), who found that the peak excretion was 2–10 h post-exposure, with a mean half-life of 4 h (range 2.2–7 h). Johanson *et al.* (1986) noted that there was a 10-fold

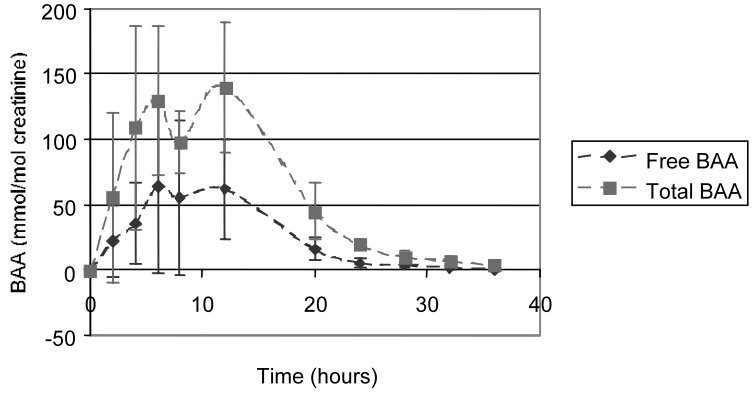

Figure 1. Mean (±SD) urinary excretion of free and total 2-butoxyacetic acid (BAA) after exposure to 50 p.p.m. 2-butoxyethanol for 2 h ($n = 4$).

difference in the excretion rates of free 2-butoxyacetic acid between volunteers. Such a disparity is also seen in Figure 1. Similarly, Johanson *et al.* (1986) reported a four-fold difference between volunteers in the total excreted dose of free 2-butoxyacetic acid. In our study the difference in the total excreted dose for free 2-butoxyacetic acid in urine is also large (five-fold), but when total 2-butoxyacetic acid is measured, the difference is reduced to two-fold. These findings highlight the benefit of using total 2-butoxyacetic acid in urine to take into account inter-individual variations in metabolism.

Both uncorrected ($\mu mol\,l^{-1}$) and creatinine-corrected ($mmol\,mol^{-1}$ creatinine) results show a similar elimination profile (Figure 2) and correlate well with the excretion rate ($r = 0.92$ for both). The uncorrected and creatinine-corrected results show a dip in excretion at 6 h post-exposure, but this dip is not reflected in the excretion rate. The uncorrected results show less variation between volunteers (CV 34% compared with 61% for creatinine-corrected results).

Measuring free 2-butoxyacetic acid in urine (as has been done in most occupational surveys) could lead to considerable error in exposure estimates due

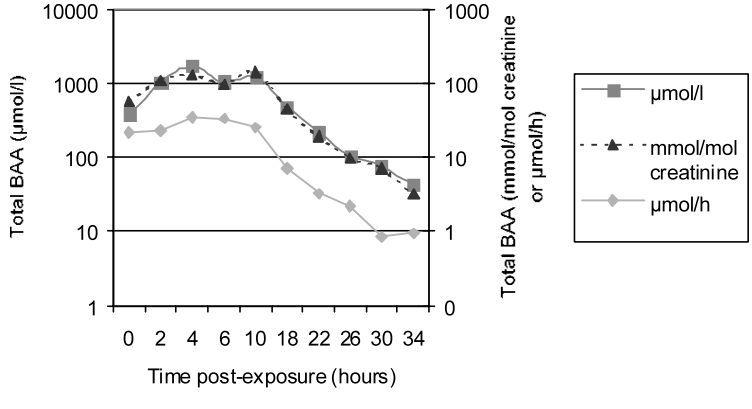

Figure 2. Mean uncorrected and creatinine-corrected urinary excretion and excretion rate of total 2-butoxyacetic acid (BAA) after exposure to 50 p.p.m. 2-butoxyethanol for 2 h ($n = 4$) (error bars are excluded for clarity).

to individual differences in conjugation. Using hydrolysed ('total') 2-butoxyacetic acid reduces this variation because hydrolysis removes inter- and intra-individual differences in the glutamine conjugation. Previous work by Rettenmeier *et al.* (1993) and Sakai *et al.* (1994) showed that conjugation can account for between 16 and 92% of the total 2-butoxyacetic acid excreted in urine. Figure 3 shows the correlation between free and total 2-butoxyacetic acid in urine from 48 workers with occupational exposure to 2-butoxyethanol, with a 1:1 correlation (assuming no conjugation) indicated by a dashed line. The results show that, while a few workers ($n = 6$) show no conjugation (i.e. their results lie on the 1:1 line), the majority of workers show some degree of conjugation (i.e. their 'total' 2-butoxyacetic acid results are greater than their free 2-butoxyacetic acid levels). Apart from the non-conjugators, the extent of conjugation appears to increase markedly at levels above 50 mmol mol^{-1} creatinine. This may indicate activation of the conjugation pathway. Previous evidence of non-conjugation has not been reported. Our data were obtained from many more individuals than previously reported (Rettenmeier *et al.* 1993, $n = 6$; Sakai *et al.* 1994, 22 samples but only six individuals; Corley *et al.* 1997, $n = 6$). With small sample numbers ($n = 6$) and non-conjugators being in a minority (12%), it is not surprising that these previous studies did not identify any non-conjugators. Rettenmeier *et al.* (1993) did suggest that saturation of the glutamine pathway had occurred in one sample where the concentration of 2-butoxyacetic acid exceeded 6 mM; however, the concentrations of the non-conjugators in Figure 3 are lower than this (maximum ~ 2 mM), so the absence of conjugation is more likely to indicate biological variation in conjugation via the glutamine pathway. Our data supports activation of the conjugation pathway at higher exposure levels (above 50 mmol mol^{-1} creatinine) for the majority of

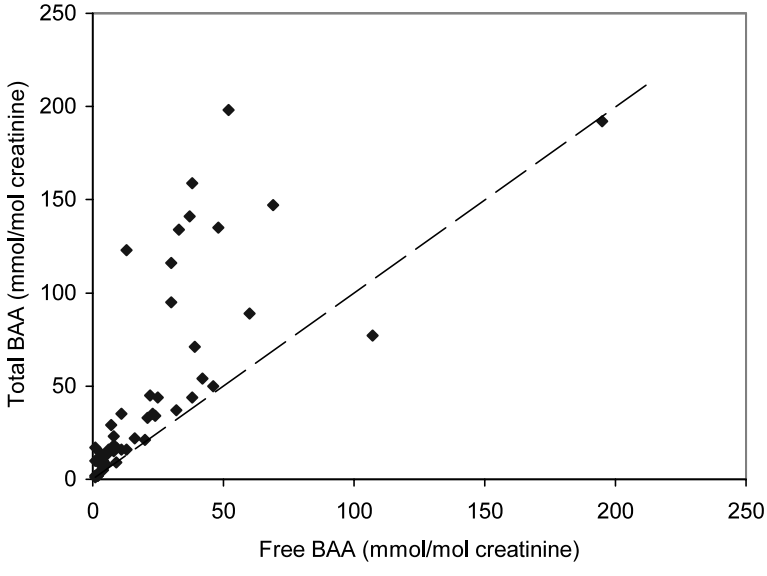

Figure 3. Correlation between free and total 2-butoxyacetic acid (BAA) in urine in 48 exposed workers. The dashed line indicates a 1:1 correlation, assuming no conjugation.

workers, rather than saturation, indicating that free 2-butoxyacetic acid levels would underestimate exposure at higher levels.

Using a similar methodology to Rettenmeier *et al.* (1993) and Sakai *et al.* (1994), the correlation between total 2-butoxyacetic acid and conjugated 2-butoxyacetic acid in the 48 exposed workers was 0.80 (least squares regression), with a relationship of $BAA_{conj} = 0.57\ (BAA_{total}) - 3.2$). Conjugated 2-butoxyacetic acid was determined by subtracting free from total 2-butoxyacetic acid. From this correlation, an estimated 57% (95% confidence interval 44–70%) of the total 2-butoxyacetic acid is excreted in the conjugated form, which agrees well with previously published data (Rettenmeier *et al.* 1993, 59%; Sakai *et al.* 1994, 64%; Corley *et al.* 1997, 67%).

The multiple samples from our volunteer study show that the degree of conjugation also varies within an individual. Analysis of samples from each of the four volunteers in this study showed that the percentage of conjugated 2-butoxyacetic acid could vary within an individual from nearly 0 to 100%. This variation seemed independent of the time of day (Figure 4) or the urine pH (data not shown). Despite the variation in the ratio of conjugated to total 2-butoxyacetic acid in each volunteer, there was good correlation overall between conjugated and total 2-butoxyacetic acid ($r = 0.70–0.96$).

Conclusions

The measurement of 2-butoxyethanol itself, in blood or breath, poses both sample-collection and analysis difficulties and is an unsuitable biomarker for assessing 2-butoxyethanol exposure. It is too polar and involatile to allow sufficient sensitivity for breath analysis to monitor low levels of exposure. When measuring 2-butoxyethanol in blood, localized high concentrations can occur, making sampling and interpretation of results difficult. 2-Butoxyacetic acid is a more suitable marker.

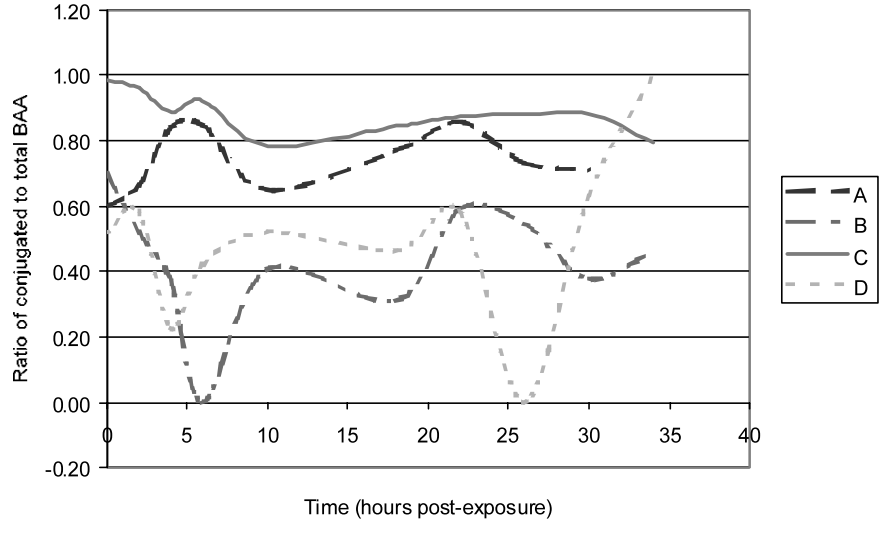

Figure 4. Variation in the proportion of conjugated 2-butoxyacetic acid (BAA) over time in four volunteers (A, B, C and D) after exposure to 50 p.p.m. 2-butoxyethanol for 2 h.

This can be measured in blood, although the levels found are much lower than in urine and, in the UK at least, non-invasive methods are preferred for occupational biological monitoring. Thus urinary 2-butoxyacetic acid is the most suitable marker for biological monitoring.

Free 2-butoxyacetic acid may be a good marker of risk as this is the active metabolite that causes haemolytic anaemia. However, total 2-butoxyacetic acid may be a better marker of exposure to 2-butoxyethanol. Since occupational exposures are likely to be much lower than the levels that cause haemolytic anaemia, it is more appropriate to use a marker of exposure rather than a marker of risk. Historical guidance values based on free 2-butoxyacetic acid will include samples containing both conjugated and non-conjugated 2-butoxyacetic acid. A guidance value derived from the relationship between environmental air 2-butoxyethanol and urine 2-butoxyacetic acid will be biased by the more numerous conjugated samples. As a result, non-conjugators are at greater risk since for a given exposure they will have higher free 2-butoxyacetic acid values (the active metabolite). When measuring 2-butoxyacetic acid in urine, measurement of only free 2-butoxyacetic acid may underestimate the absorbed dose of many workers, as the present study has demonstrated that a majority of workers demonstrate some conjugation of 2-butoxyacetic acid. The extent of variation within workers (Figure 4) suggests that this variation may not be due to different polymorphisms (as is the case for acetylation, with slow and fast acetylators being genetically determined), but may be the result of other factors. It may be affected by glutamine levels; glutamine is produced by the body and is also present in diet, and depletion of glutamine levels can be caused by injury, stress and disease. Since the extent of conjugation in any given sample cannot be predicted, use of total 2-butoxyacetic acid is further justified in order to give an estimate of the absorbed dose. Despite the variation, there is a good correlation between conjugated and total 2-butoxyacetic acid ($r = 0.80$, $p < 0.0001$), indicating that, on average, 57% of the total excreted 2-butoxyacetic acid is conjugated (95% confidence interval 44–70%). Such a conversion factor could be applied to previous occupational surveys that have looked only at free 2-butoxyacetic acid in order to determine potential guidance values for total 2-butoxyacetic acid.

The volunteer study presented here does not support the concept that, at occupational exposure limits, conjugation may be saturated. The 'whole body' study (50 p.p.m. exposure) gave a significantly higher ($p = 0.07$) proportion of conjugated 2-butoxyacetic acid than the 'skin only' study (equivalent to ~ 5 p.p.m. exposure) in the same volunteers. This is in agreement with the occupational samples, which suggest conjugation may be activated above a certain exposure level, although some non-conjugation is still observed.

Although the elimination half-life of 2-butoxyacetic acid in urine is only about 6 h, peak excretion does not occur until 6–12 h post-exposure. With repeated exposures throughout the working week, accumulation of 2-butoxyacetic acid is therefore possible, indicating that urine samples should be taken post-shift towards the end of the working week. Both uncorrected and creatinine-corrected results are well correlated with excretion rate, although uncorrected results appear to give less

inter-individual variation (based on four volunteers). Existing guidance values use both corrected (HSE 2002) and uncorrected (DFG 2002) values.

Using total 2-butoxyacetic acid as a marker, skin absorption of 2-butoxyethanol vapours accounts for about 11% of the total body burden. This is higher than for other chemicals (butanone, methoxy-2-propanol, tetrahydrofuran, toluene and xylene) previously studied using the same methodology (Brooke *et al.* 1998). It is interesting that 2-butoxyethanol appears, *in vivo*, to be absorbed better than methoxy-2-propanol, when the reverse has been shown *in vitro* (Dugard *et al.* (1984)). The absorption of 2-butoxyethanol vapours under various environmental conditions has been studied further (Jones *et al.* 2003), indicating that increased temperature and humidity can significantly increase dermal absorption.

In conclusion, we suggest that total 2-butoxyacetic acid in urine is the biomarker of choice for assessing occupational exposure to 2-butoxyethanol. Samples should be collected post-shift towards the end of the working week.

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