Case studies of hydrogen sulphide occupational exposure incidents in the UK

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HIGHLIGHTS

- Three case studies of industrial incidents involving hydrogen sulphide are presented.
- We demonstrate the use of thiosulphate measurements in blood and urine.
- Appropriate sample collection and storage are important factors.
- The role of biological monitoring in such incidents is discussed.

ABSTRACT

The UK Health and Safety Executive has investigated several incidents of workplace accidents involving hydrogen sulphide exposure in recent years. Biological monitoring has been used in some incidents to determine the cause of unconsciousness resulting from these incidents and as a supporting evidence in regulatory enforcement. This paper reports on three case incidents and discusses the use of biological monitoring in such cases. Biological monitoring has a role in identifying hydrogen sulphide exposure in incidents, whether these are occupational or in the wider environment. Sample type, time of collection and sample storage are important factors in the applicability of this technique. For non-fatal incidents, multiple urine samples are required at two or more time points between the incident and 15 h post-exposure. For routine occupational monitoring, post-shift samples should be adequate. Due to endogenous levels of urinary thiosulphate, it is likely that exposures in excess of 12 ppm for 30 min (or 360 ppm/min equivalent) would be detectable using biological monitoring. This is within the Acute Exposure Guideline Level 2 (the level of the chemical in air at or above which there may be irreversible or other serious long-lasting effects or impaired ability to escape) for hydrogen sulphide.

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1. Introduction

Hydrogen sulphide is a toxic gas generated by non-specific and anaerobic bacterial reduction of sulphates and sulphur-containing organic compounds. Natural sources include crude petroleum, natural gas, volcanic gases and hot springs. It can also be found in groundwater and released from stagnant or polluted waters and manure or coal pits. The principal industrial source of hydrogen sulphide is recovery as a by-product in the purification of natural and refinery gases. It is also a by-product of pulp and paper manufacturing and carbon disulphide production. It is used as an intermediate in manufacturing processes (e.g. sulphuric acid) (WHO, 2003). In the UK, regulations are in force requiring storage of slurry (including manure) in certain areas to prevent water pollution (DEFRA, 2010). Similarly, the UK Government is committed to increasing energy production through anaerobic digestion (DEFRA, 2011). These factors have increased potential exposures to hydrogen sulphide in the UK.

Human exposure to exogenous hydrogen sulphide is principally via inhalation with rapid absorption. Hydrogen sulphide is metabolised through three pathways: oxidation, methylation, and reactions with metalloproteins or disulphide-containing proteins. Oxidation in the liver is the major detoxification pathway, forming thiosulphate, which is then converted to sulphate and excreted in the urine. The methylation pathway also serves as a detoxification route. The toxicity of hydrogen sulphide is a result of its reaction with key metabolic metalloenzymes. In the mitochondria, cytochrome oxidase (the final enzyme in the respiratory
chain) is inhibited by hydrogen sulphide. This disrupts the electron transport chain and impairs oxidative metabolism which particularly impacts nervous and cardiac tissues (both are tissues with high oxygen demand and rely on oxidative metabolism). In the central nervous system, this effect may result in unconsciousness or even death from respiratory arrest (WHO, 2003). High flow oxygen is generally used to treat victims of hydrogen sulphide poisoning (Gresham, 2014) although other treatments such as hyperbaric oxygen and parenteral administration of a methaemoglobin inducing agent (such as sodium nitrite) have also been reported (Costigan, 2003; Belley et al., 2005).

Hydrogen sulphide is acutely toxic with fatalities associated with concentrations in excess of 500 ppm. It has a very low odour threshold (0.008 ppm) but odour perception is lost at concentrations of 150–250 ppm (WHO, 2000), adding to the danger of high level exposures as they may not be recognised, by smell, by the individual. In Europe, there is a workplace exposure limit (8 h TWA) of 5 ppm (HSE, 2011; SCOEL, 2007) with a short-term (15-min) exposure limit of 10 ppm.

Hydrogen sulphide has previously been reported as a causal agent of unconsciousness and death in a number of occupational exposure incidents (Kage et al., 2002, 2004). In the UK it has been reported (Costigan, 2003) that around 125,000 workers in the UK are potentially exposed to hydrogen sulphide in work related to the treatment of sewage, effluent waste and farm slurry. In the offshore oil and gas industries about 3000 workers are potentially exposed. The UK Health and Safety Executive has investigated several incidents of workplace accidents involving hydrogen sulphide exposure from slurry pits, animal rendering plants and biodigester facilities in recent years. The increased prevalence of biodigesters and slurry storage may indicate an increased likelihood of further incidents in the future. Here we report three case studies using biological monitoring to determine hydrogen sulphide exposure.

2. Material and methods

Blood or urine thiolsulphate determination was carried out according to the method of Kage et al. (1991). Briefly, samples (200 µl) were buffered with ascorbic acid (200 mM, 50 µl) and 5% sodium chloride (50 µl) then derivatised using pentafluorobenzyl bromide (20 mM in acetone, 500 µl) and extracted into iodicine ethyl acetate solution (25 mM, 2 ml) to form bis(pentafluorobenzyl) disulphide. Tribromobenzene was used as an internal standard. Analysis was by gas chromatography–mass spectrometry (positive electron ionisation) using selected ion monitoring (m/z 426 for the thiolsulphate derivative). Aliquots (1 µl) were injected (220 °C, splitless) onto a BP-5 equivalent column (30 m × 0.32 mm i.d., 1 µm film) with a helium flow of 1 ml/min. The oven temperature was held at 100 °C for 2 min then ramped at 10 °C/min up to 220 °C, where it was held for 5 min. Calibration standards were prepared in blood or urine, as appropriate, and extracted as per the samples. The calibration curves were linear from 0 to 600 µmol/l (least squares regression > 0.99) and quality control samples were within the expected range showing a coefficient of variation of 12%. The detection limit was 1 µmol/l. Urine samples were also analysed for creatinine content using the alkaline picrate reaction (Cocker et al., 2011)

3. Results

3.1. Case 1

Two workers were admitted to hospital after collapsing in an enclosed waste intake area of an animal rendering plant. One was unconscious on admission. Both provided urine samples whilst at the hospital – worker 1 (male, 53 years old) approximately 9 h after the incident, worker 2 (male, 54 years old) at an unknown time (but apparently the same day) by catheter as he was still unconscious. The urine sample for worker 1 contained 326 µmol/l thiolsulphate (23 mmol/mol creatinine), which is consistent with the levels seen in other survivors of reported incidents of hydrogen sulphide exposure where samples have been taken between 2 and 15 h of the incident (Kage et al., 1997, 2002). Worker 2’s result (10 µmol/l, 2 mmol/mol creatinine) was within previously reported background levels (Kangas and Savolainen 1987; Chwatkow and Bald, 2009) however it is not clear when the sample was collected in relation to the incident. It is possible that, if he was exposed, it might take a couple of hours for his thiolsulphate level to exceed background levels (as demonstrated by a volunteer study (Kangas and Savolainen, 1987)); so if the sample was taken shortly after the incident, the sample may not reflect the extent of his exposure to hydrogen sulphide. Equally, if the sample had been taken later, the level of thiolsulphate may already have reduced to background levels. There is previously reported, (Kage et al., 1997) a case (in which a man lost consciousness due to hydrogen sulphide exposure and subsequently recovered) where the urinary thiolsulphate level was less than 3 µmol/l when the sample was taken 15 h after the incident.

There was evidence that worker 1 had been exposed to hydrogen sulphide in sufficient amounts to cause a feeling of unwellness or even unconsciousness. The sample of worker 2 did not demonstrate evidence of hydrogen sulphide exposure but this does not exclude the possibility of exposure due to the unknown timing of sample collection.

3.2. Case 2

A chicken waste rendering plant had a blocked condenser connected to a storage vessel. On releasing the blockage, an emission of gas (suspected to contain hydrogen sulphide) was released knocking three workers unconscious. All three workers were taken to hospital, two were subsequently released and one spent time in intensive care before being released. Blood samples were obtained from two of the workers (both male, ages unknown) but were not detectable for thiolsulphate. This is in agreement with previous reports where blood thiolsulphate is not detected in survivors of hydrogen sulphide incidents. Unfortunately, in this case, it was not possible to obtain urine samples. Samples of the chicken waste showed considerable potential for hydrogen sulphide generation at the sterilising temperature used (−120 °C).

3.3. Case 3

One urine sample and one blood sample were received from a fatality (male, age unknown) involving a biodigester, where hydrogen sulphide was a suspected toxic agent. The urine sample was below the detection limit for thiolsulphate. The blood sample had a detectable thiolsulphate level of 22 µmol/l.

The blood level reported is at the lower end of the scale of previously reported fatalities (25–230 µmol/l) but definitely indicates significant hydrogen sulphide exposure – sufficient to cause unconsciousness, and possibly fatal poisoning. No thiolsulphate was detected in urine, which is consistent with literature reports of sudden death caused by hydrogen sulphide (Kage et al., 2002) whereas survivors of hydrogen sulphide poisoning incidents tend to have raised urinary thiolsulphate levels in the hours following the incident as thiolsulphate is excreted.

It can therefore be concluded that the results of the thiolsulphate analysis from blood and urine samples are consistent with acute hydrogen sulphide poisoning causing death rapidly. However, it should be noted that these analyses were conducted some nine months after the incident occurred. The samples were
previously stored by a third party and thought to have been refrigerated. There have been reports that sulphide can be generated post-mortem in blood and other tissues (Nagata et al., 1990) and this can then be converted to thiosulphate within the sample (Tsuge et al., 2000). However, it has also been reported that refrigerated storage suppresses such post-mortem sulphide production (Nagata et al., 1990) which would therefore support the conclusion of acute hydrogen sulphide poisoning in this case.

4. Discussion

Mean background levels of thiosulphate in urine from people with no known overt exposure to thiosulphate have been reported as 2.9 mmol/mol creatinine (standard deviation of 2.5 in a group of 29 individuals (Kangas and Savolainen, 1987)). Although, this is a limited dataset, it would tentatively suggest that a reference range for the general population might be approximately <7.9 mmol/mol creatinine (taking 95th percentile as the mean plus two standard deviations). Another study reported background levels of 1.36–4.89 mmol/mol creatinine (N = 13, (Chwatto and Bald, 2009)).

A controlled human volunteer study where a volunteer was exposed to 18 ppm hydrogen sulphide for 30 min (Kangas and Savolainen, 1987) has also been reported. The concentration of thiosulphate in urine increased after exposure, reaching a maximum of 30 mmol/mol creatinine at 15 h. Levels had returned to normal by 17 h. However, no samples were taken between 5 and 15 h after exposure as this was overnight. It is therefore likely that the actual maximum concentration in urine is between 5 and 15 h. Because the morning void sample had accumulated thiosulphate over the preceding 10 h and the following sample (17 h) was back in the general population range, no estimation of excretion half-life is possible. A study (Fares et al., 2011) looking at sodium thiosulphate pharmacokinetics indicates a serum half-life of roughly 40 min.

Raised urinary thiosulphate levels in survivors have been used to demonstrate hydrogen sulphide exposure incidents (Table 1). Nikkanen and Burns (2004) reported the case of an adolescent who was rendered unconscious whilst cleaning a reoxygenation tank in a fish hatchery. He was revived and then taken to hospital – his urinary thiosulphate was measured as 79 mmol/mol creatinine. Kage et al. (2002) reported an incident at an industrial waste pit where three men died after entering a pit (one of whom died 22 days after the incident) and one worker survived. The delayed fatality and the survivor both had detectable levels of thiosulphate in urine in samples taken 2 h after the incident (1225 and 262 μmol/l, respectively, ~102 and ~22 mmol/mol creatinine – conversion assumes a mean creatinine concentration of 12 mmol/l, (Cocker et al., 2011)). Kage et al. (1997) reported an incident where four workers lost consciousness in an underground tank in a factory producing regenerated paper, all four workers recovered. Urinary thiosulphate levels ranged from 120 to 430 μmol/l (~10–36 mmol/mol creatinine), in samples taken 6 h post-incident and from <3 to 390 μmol/l (~0.3–33 mmol/mol creatinine), in samples taken 15 h post-incident.

There are several reports in the literature of blood thiosulphate levels being detected after hydrogen sulphide fatalities (Table 1). The levels reported range between 25 (Kage et al., 1997) and 230 μmol/l (Kage et al., 2004). Rabbits that received a fatal dose of hydrogen sulphide (500–1000 ppm for up to 30 min) gave blood thiosulphate levels of 53–119 μmol/l (Kage et al., 1992), which is in good agreement with the human fatality studies. Survivors of poisoning incidents are not reported to have detectable blood thiosulphate levels, as the body rapidly clears the blood, nor are the general population.

It has therefore been demonstrated, both within the case studies presented here and in the literature, that blood and/or urinary thiosulphate measurements can be useful in determining hydrogen sulphide as a potential cause of fatality or unconsciousness. The analysis is sufficiently sensitive to discriminate exposures from control samples and has reasonable specificity, if storage conditions are controlled. However, there are certain considerations that need to be taken into account in order to get the most useful information from such analyses. First, the type of sample required will depend on the condition of the workers – if they are survivors of incidents then urine samples are most appropriate as the body will rapidly clear any thiosulphate from the blood. In the case of fatalities (to determine likely cause of death or to assist in any related investigation), blood samples are most appropriate. Urine samples may be useful as additional samples to ascertain whether death was instantaneous or delayed after a period of unconsciousness, especially if the worker was not discovered until sometime after the incident. Secondly, the timing of the sample relative to the incident is important for detecting exposures in survivors. It has been shown in volunteers (Kangas and Savolainen, 1987) and workers (Kage et al., 1997) that samples taken more than 15 h after an incident are likely to be in the general population range. It is important therefore, to obtain urine samples from victims of potential hydrogen sulphide incidents within 15 h. A human volunteer study (Kangas and Savolainen, 1987) showed that after a 30 min exposure to hydrogen sulphide, raised urinary thiosulphate levels were not detected until 2 h after the start of exposure whereas an animal study (Kage et al., 1992) demonstrated a maximal urinary thiosulphate concentration at 1 h post exposure (hydrogen sulphide exposures were very much higher in this study, 100–200 ppm). It may therefore be prudent to take multiple urine samples where a hydrogen sulphide incident is suspected – as soon as possible after the incident and further samples between 2 and 15 h post-exposure. Such samples may not capture the ‘maximal’ excretion (which might be expected at 15 h post exposure according to the volunteer reported (Kangas and Savolainen, 1987) although, no samples were taken between 5 and 15 h, being overnight) but would be likely to capture any increase in urinary thiosulphate levels, sufficient to determine hydrogen sulphide as a likely causal agent in the incident. The use of multiple, timed samples may also assist in reconstructing the exposure; a linear relationship between time post-exposure and urinary thiosulphate levels has been demonstrated (Kangas and Savolainen, 1987). Finally, storage conditions of post-mortem samples are important. As demonstrated in one of the case reports here, it is not unusual to receive post-mortem samples some months after the death has occurred. If samples have not been appropriately stored then bacterial action during storage may confound the findings of the analysis. The use of thiosulphate as a biomarker in assisting clinical diagnosis, and therefore treatment, is unlikely due to the current limited availability of this analysis in laboratories and the time taken to generate a result (although, theoretically, a screening result could be available within an hour or so if facilities were available at the relevant hospital).

Table 1

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<tr>
<th>Fatality</th>
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<td>Blood</td>
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<td>This paper</td>
<td>22</td>
</tr>
<tr>
<td>(Nikkanen and Burns, 2004)</td>
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<td>(Kage et al., 2002)</td>
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<td>110–230</td>
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N.A., not analysed.

* 1225 μmol/l detected in delayed fatality (22 days after incident).
There are no literature reports of using biological monitoring routinely to assess occupational exposure to hydrogen sulphide. Acute, high level exposures can generally be prevented by using real-time gas sensors with appropriate alarm levels; however, there is an argument for monitoring workers exposed to more chronic, low-level concentrations. There have been a number of papers from Bhambhani et al. looking at the physiological consequences of hydrogen sulphide exposure at the current exposure limits (Bhambhani and Singh, 1991; Bhambhani et al., 1997). These have demonstrated uncertainty around anaerobic respiration and increased lactic acid production at such exposure levels. Although, these studies showed that the current exposure limits were acceptable for fit young adults, there is a possibility of effects in older, less fit workers or in susceptible groups in the general population (children, the elderly, those with pre-existing medical conditions etc.).

A volunteer study (Kangas and Savolainen, 1987) demonstrated a linear relationship between hydrogen sulphide exposure (expressed as \( \mu \text{mol} \times \text{min}/\text{l} \) and urinary thiocyanate using four exposures between 8 and 30 ppm for 30–45 min each. The resulting correlation suggests that urinary thiocyanate measurements would have sufficient sensitivity to monitor exposures as low as 360 ppm/min (using 10 mmol/mmol creatinine urinary thiocyanate as the lowest level indicating exogenous exposure). For workers exposed occupationally over an 8 h shift, this would equate to hydrogen sulphide concentrations as low as 1 ppm (8 h TWA). For general population or incident exposures, a 30 min exposure to 12 ppm should be discernible in a maximal urine sample. This is well within the Acute Exposure Guideline Level 2 (the level of the chemical in air at or above which there may be irreversible or other serious long-lasting effects or impaired ability to escape) for hydrogen sulphide (US EPA, 2012) of 32 ppm for 30 min. Biological monitoring could have a role if used in general population exposure incidents to reassure complainers that levels experienced were not harmful (it is likely that complaints would arise from the public at low levels of exposure due to the low odour threshold). Further data on the correlation between hydrogen sulphide exposure and urinary thiocyanate levels would be helpful in aiding such risk communication.

In conclusion, biological monitoring has a role in identifying hydrogen sulphide exposure in incidents, whether these are occupational or in the wider environment. Sample type, time of collection and sample storage are important factors in the applicability of this technique. For non-fatal incidents, multiple urine samples are recommended at two or more time points between the incident and 15 h post-exposure. For routine occupational monitoring, post-shift samples should be adequate. Due to endogenous levels of urinary thiocyanate, it is likely that exposures in excess of 12 ppm for 30 min (or 360 ppm/min equivalent) would be detectable using biological monitoring.

**Acknowledgements**

This publication describes work funded by the Health and Safety Executive (HSE), its contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy.

**References**


**Conflict of interest**

The author declares that there is no conflict of interest.

**Transparency document**

The Transparency document associated with this article can be found in the online version.