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## **IS PHOSPHINE A COMPONENT OF CONCERN IN RUMEN GAS IN CATTLE?**

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### **ABSTRACT**

Studies on anaerobic biological systems indicate that they may produce phosphine (PH<sub>3</sub>) as well as methane. Such systems include the digestive tract of ruminants. As part of a project to monitor environmental levels of PH<sub>3</sub> produced by grain fumigation and other possible sources of the gas, the authors set out to determine the PH<sub>3</sub> levels of rumen gas in cattle.

Samples of rumen gas were taken from lactating dairy cattle and analysed shortly afterwards by gas chromatography. Some samples were taken from cattle fitted with a rubber fistula, permanently connecting the rumen to the skin and others were drawn directly from the rumen of unfistulated cattle.

Phosphine was not detected above the limit of detection (0.01 ppb) for the analytical method in any sample taken from these cattle. This indicates that PH<sub>3</sub> is not a significant component in the rumen of lactating dairy cattle and would not present a hazard to workers handling cattle. There were differences between some of the unidentified components of the fistulated and unfistulated cattle, that were thought to be caused by gas interchange through the fistula, which changed the anaerobic status of the rumen in these cattle.

### **INTRODUCTION**

In 1989, Garry *et al.*, indicated that humans exposed to phosphine (PH<sub>3</sub>) had more chromosomal changes than those in a control group. After a review of data then available on the effects of PH<sub>3</sub> exposure, the Committee on Toxicity within the Australian National Health & Medical Research Council (NH&MRC) recommended an environmental level of 3 ppb PH<sub>3</sub> (Anon. 1992). The Committee set a short-term level of 13 ppb that recognised the difficulty of routine measurement of concentrations at this low level, which itself is at the lower end of measurement for some electrochemical devices, paper tape devices and detector tubes.

An investigation into environmental levels of PH<sub>3</sub> produced by grain fumigation in Australia (Pratt 1999) showed average dispersion to below the proposed environmental level was reached about 25 m down-wind of large sheds being fumigated using the SIROFLO<sup>□</sup> technique, where the in-grain concentration was 50 ppm.

Gassmann and Glindemann (1993) indicated that anaerobic biological systems, which produce methane, also produce  $\text{PH}_3$  and diphosphine ( $\text{P}_2\text{H}_4$ ). They also suggested that  $\text{PH}_3$  may play a significant role in the global phosphorus cycle and possibly poses a health risk to humans.

As part of an investigation into environmental sources of  $\text{PH}_3$  and whether these sources might exceed the proposed environmental level, this paper reports work that examined one possible source of  $\text{PH}_3$  in the biosphere, the rumen gas of lactating dairy cattle.

### MATERIALS AND METHODS

The research was carried out at Ellenbank Dairy Research station in Victoria Australia. Lactating dairy cattle were tested because they were on a diet high in phosphate. Some of the cattle tested were fitted with a rubber fistula, which connected the rumen to the skin, allowing easy sampling of the rumen gas by syringe. Because this procedure may have produced a contaminated sample, or the anaerobic status of the rumen may have been compromised, five cattle, without fistula, were sampled directly through the wall of the rumen. These samples were taken by inserting a small cannula directly through the skin into the rumen and withdrawing a 1 L sample of gas by syringe, which was transferred to nitrogen flushed evacuated Tedlar<sup>®</sup> gas sampling bags for subsequent analysis. Sub-samples of 100 mL of rumen gas were then analysed by gas chromatography after passing them through a filter of sodium hydroxide to dry them before the  $\text{PH}_3$  was concentrated onto a cryotrap cooled in a bath of salted ice in water ( $\square -18^\circ\text{C}$ ). This temperature was used, instead of a dry-ice/ethanol bath ( $\square -80^\circ\text{C}$ ) used for ambient air samples, to allow the high concentration of methane present to be flushed off the trap before heating the trap to flush the contents onto a gas chromatograph column. This prevented the large amount of methane present in samples from interfering with the response of the thermionic detector used to detect the  $\text{PH}_3$ . The limit of detection for the method used was less than 0.01 ppb of  $\text{PH}_3$ . A prepared gas standard of 1 ppb  $\text{PH}_3$  in air was used to establish the retention time and sensitivity of the detector for  $\text{PH}_3$ , and for spiking samples.

### RESULTS

Although there appeared to be differences between the gas content of cattle fitted with a fistula and those cattle not fitted with a fistula, no  $\text{PH}_3$  was detected in any sample from either source above the detection limit of 0.01 ppb. An unidentified peak, shown in Fig. 1, may have been  $\text{P}_2\text{H}_4$ , as its relative retention time was similar to that of a trace for  $\text{P}_2\text{H}_4$  published by Gassmann and Glindemann (1993). However, we did not have a sample of this gas to check for this possibility. Figure 1 shows typical traces of samples taken directly from cattle without a fistula. Trace 1b shows a sample spiked with a small amount of  $\text{PH}_3$ , which shows that the unidentified peak is not  $\text{PH}_3$ . Other samples from air above a manure sludge pit and gas collected from a bottle of manure allowed to ferment overnight did not appear to have  $\text{PH}_3$  above the limit of detection.

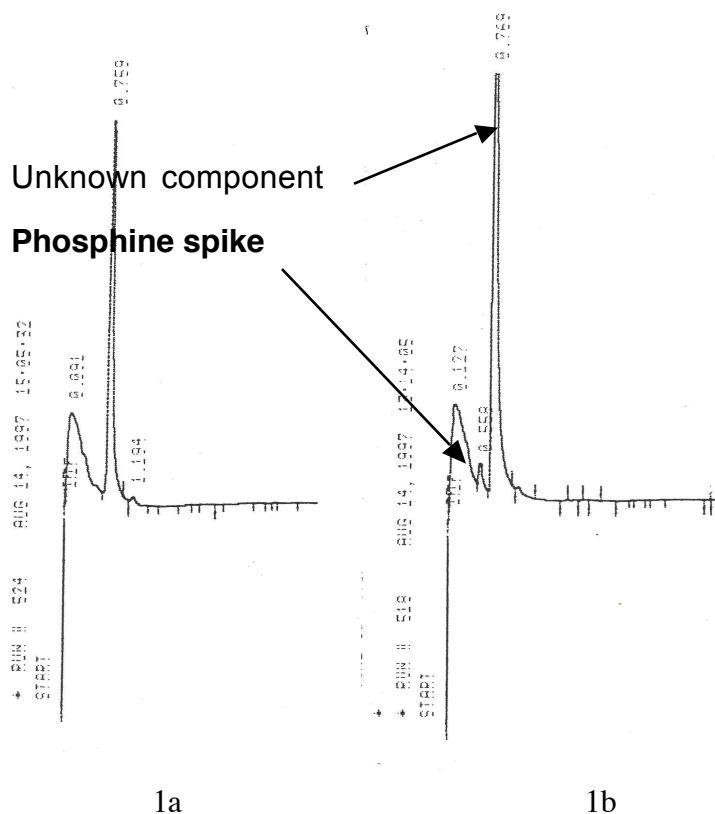


Fig. 1. GC Traces of gas samples taken directly from the rumen of lactating dairy cattle and concentrated on a cold trap. Trace 1b was spiked with a small amount of phosphine

### DISCUSSION

The unidentified peak may be  $P_2H_4$ . However we were not able to definitively verify this possibility. Gassmann and Glindemann (1993), show data for  $PH_3$  and  $P_2H_4$  concentrations in the digestive tracts of cattle and pigs and reported levels for  $PH_3$  from 0.003 ppb (below the limit of detection for the analytical technique used here) to approximately 1 ppb. The level of  $P_2H_4$  exceeded that of  $PH_3$  in

some of their incubated samples indicating a difference in the way the bacteria used the available phosphate. In addition, the presence of  $\text{PH}_3$  and  $\text{P}_2\text{H}_4$  is thought to be directly associated with biogenic methane production (Gassmann and Glindemann, 1993). However, Jenkins *et al.*, (2000) recently suggested that  $\text{PH}_3$  and methane are produced by different bacteria than those responsible for methanogenesis.

Though the rumen gas components of fistulated and unfistulated cattle appeared to differ, no  $\text{PH}_3$  was detected at levels above the limit of detection for the method. Given that any flatus would be diluted in air, this would suggest that the quantity of  $\text{PH}_3$  produced in the rumen of grazing dairy cattle would not present a hazard to workers occupationally exposed to the flatus of cattle.

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