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Note

The determination of metaldehyde in biological material by head-space gas chromatography

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Metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetroxocane), the tetramer of acetaldehyde has been used in horticulture and agriculture since the discovery of its molluscicidal properties in 1936[1]. A survey of pesticide usage in agriculture and horticulture in Scotland conducted during the period 1975–1977[2] estimated that 2.2 tons of active ingredient was used annually. The commercial formulation is prepared in pellet form and contains approximately 6% metaldehyde, while the remainder is usually a coarse bran mixture. The pellets appear to be particularly palatable to cats, dogs and many other domestic and farm animals and several cases of poisoning have been reported where animals have had access to large quantities of the formulation[3,4].

In recent years this laboratory has received tissues for analysis, usually from cats or dogs, from incidents where animal deaths may have resulted from metaldehyde ingestion. Few methods have been published for the specific analysis of metaldehyde residues in animal tissue, although a number have been reported for its determination in certain plant tissues, notably strawberries and artichokes[5], corn and bean foliage[6] and lettuce[7].

Tissue samples received by this laboratory are usually kidney, liver or stomach contents from animals which have displayed symptoms which may be consistent with metaldehyde poisoning before death. Symptoms may include hyperaesthesia, incoordination, excessive salivation, increased heart and respiratory rates, convulsions and coma[8].

The previous method used in the laboratory for detection of metaldehyde in animal tissue was based on a spot test in which the tissue sample is treated with strong acid and heated in a test tube[9]. Any metaldehyde present is converted to acetaldehyde vapour which is driven off and detected by a mixture of morpholine and sodium nitroprusside as a blue colouration on test paper[10]. Unfortunately this blue colour is unstable and quickly disappears. The limit of detection of this test varies with tissue type, approximately 30 μg of metaldehyde in the sample is necessary for a confident identification. Wildlife and other incidents thought to have been caused by metaldehyde are received sporadically. Usually they are accompanied by limited field information concerning the incidents so that fatality may be due to one of many poisons and not specifically metaldehyde.

Work was undertaken to develop a reasonably quick but reliable method for metaldehyde analysis in animal tissue. The method measures acetaldehyde by gas

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chromatography (GC) in the head-space above acidified tissue digests. The analytical work was carried out on kidney and liver tissue and on stomach contents where highest levels were likely to be present. The method was developed using tissue samples from animals dosed orally at 700 mg/kg, slightly above the quoted LD_{50} (oral) of 630 mg/kg, and at 100 mg/kg. It was hoped that these studies would show the likely residue levels which would occur as a result of animals receiving sub-lethal and lethal doses of metaldehyde. Rats were used throughout this study because of their availability, ease of handling and known toxicological data. Animals were killed humanely at various intervals after dosing and liver, kidney and stomach contents were removed for analysis.

EXPERIMENTAL

A gas chromatograph (Perkin-Elmer Sigma 2) equipped with a flame ionization detector was used throughout. A 1.8 m × 4 mm I.D. glass column packed with Porapak Q 80–100 mesh (Waters Assoc.) was maintained at 160°C with a nitrogen carrier gas flow-rate of 50 ml min^{-1}, detector and injector temperatures were maintained at 320°C and 220°C respectively. A reporting integrator (Hewlett-Packard 3390A) was used for peak area measurement and recording retention times. Glass bottles (Pyrex Reagent Bottles, 100 ml) fitted with screw tops and silicone rubber septa were used as digestion vessels. The screw tops were drilled with 3-mm diameter holes allowing needle insertion of a 500-μl gas tight syringe (SGE, U.K.). The digestion was carried out in a glycerol bath held at 130°C.

Biological samples

Ten six-week-old rats were allowed to feed *ad libitum* for 14 days on a diet composed chiefly of ground wheat and oats. Eight were dosed orally by stomach tube with a suspension of metaldehyde in paraffin oil then allowed to feed *ad libitum*. Two of these rats were dosed at 700 mg/kg and six others at 100 mg/kg. One rat dosed at 700 mg/kg died after 1 h 50 min. The other rat dosed at 700 mg/kg, two rats dosed at the lower level and the two controls were all killed after 2 h. Of the remaining four rats dosed at 100 mg/kg, two were killed 6 h after dosing and the other two rats 24 h after dosing. Immediately after death, liver, kidney, stomach and intestinal tract were removed and deep-frozen (−18°C approximately) until analysed.

Analysis of biological samples

Deep-frozen tissue was allowed to thaw completely. Kidney and liver were finely chopped and a portion (0.5–2.0 g) was placed in a digestion bottle described above. The entire stomach contents were minced and a portion (1.0 g) taken for analysis. Sulphuric acid (6 *N*, 15 ml) was added to the digestion bottle together with a 1-ml portion of internal standard. The bottles were capped and immediately placed in a glycerol heating bath at 130°C for 10 min, during which time the contents were mixed by occasional swirling by hand. The head-space was sampled (250 μl) using a pre-heated gas-tight syringe and injected directly into the gas chromatograph.

Metaldehyde standard solutions were prepared daily by dissolving metaldehyde (Hopkin and Williams) in chloroform. A calibration curve was also prepared daily by the addition of aliquots of the standard metaldehyde solutions to the diges-

tion bottles. The metaldehyde standards were evaporated to dryness by a gentle stream of compressed air before addition of the sulphuric acid and dichloromethane internal standard. Stock solutions of the internal standard, dichloromethane (2.2 mg/ml, glass-distilled), were prepared each day by dissolving in de-ionised water and storing at 4°C when not in use. the internal standard was prepared for use by a ten-fold dilution of the stock solution. Quantitation of metaldehyde in biological samples was determined from the plot of acetaldehyde–dichloromethane peak-area ratio *versus* metaldehyde concentration.

RESULTS AND DISCUSSION

Initial experiments employing the head-space sampling method were carried out using small quantities of metaldehyde to optimise the experimental conditions providing a rapid breakdown to produce acetaldehyde. Extraneous chromatographic peaks of long retention times frequently appeared using bath temperatures of 60–80°C which could interfere with subsequent injections. Although these compounds were not identified they were thought to be polymeric compounds produced either from incomplete breakdown of metaldehyde or subsequent polymerisation of acetaldehyde. This problem was overcome by increasing the bath temperature to 130°C. This temperature reduced the time required for conversion of metaldehyde into acetaldehyde, 10 min was found to be the optimum.

In searching for a suitable compound for use as an internal standard, a number of aldehydes and alcohols were considered. However, the presence of impurities or unsuitable retention times eliminated the most likely compounds. Dichoromethane

Fig. 1. Gas chromatogram of head-space above acid-treated metaldehyde (50 μg). Peaks; A = acetaldehyde; B = dichloromethane (internal standard). GC conditions: Porapak Q (80–100 mesh) 1.8 m × 4 mm I.D. glass column, Column temperature: 160°C.

was chosen because of its availability in a highly pure state and its convenient chromatographic retention time. A typical gas chromatogram obtained by sampling the head-space above 50 μg of acid-treated metaldehyde is shown in Fig. 1.

A calibration curve was constructed using amounts of metaldehyde ranging from 10 to 1000 μg (Fig. 2). The plot of the ratio of acetaldehyde/internal standard peak areas versus amount of metaldehyde gave a straight line passing through the origin. Under these conditions this method is able to detect acetaldehyde liberated from 1 μg of metaldehyde. The level of detection can be improved considerably by using a smaller head-space volume and taking a larger sample volume, however, this leads to variation in peak areas of identical standards, probably due to errors in sampling from the relatively small head-space volume.

Recoveries of 90–93% were obtained by adding a range of amounts of metaldehyde (50–1000 μg) to finely chopped kidney (2 g) obtained from a dog known to have died from other causes. A chromatogram obtained from a kidney sample spiked at 25 mg/kg is shown in Fig. 3. Liver tissue from this dog spiked at similar levels gave recoveries of 75–78%. These values were obtained after allowing for a background contribution in the head-space above unspiked tissue. This background contribution from animal tissue is variable, depending on type and age of the tissue. Analysis of liver and kidney from a variety of animals known to have died from causes other than metaldehyde poisoning, have shown that background levels gen-

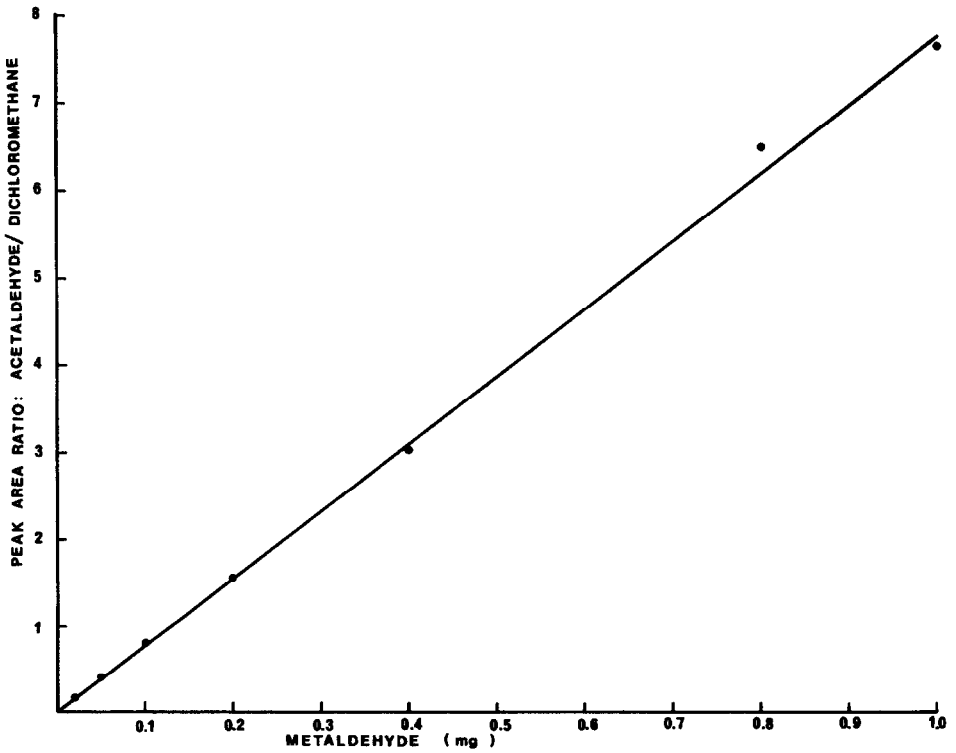

Fig. 2. Calibration constructed from head-space GC peak area ratios of acetaldehyde and internal standard dichloromethane *versus* amount of metaldehyde.

Fig. 3. Gas chromatogram of head-space above acidified dog kidney spiked with metaldehyde (50 μg/2 g = 25 mg/kg). Peaks A and B and GC conditions as in fig. 1.

erally fall within a range equivalent to 0–20 mg/kg metaldehyde. The figures in Table I for the control rats are typical background values for liver and kidney tissues. Stomach contents do not usually give rise to any measurable amount of acetaldehyde in the head-space vapour. The gas chromatogram obtained from the head-space above the acid-treated liver of one of the control rats, (No. 2) is shown in Fig. 4.

TABLE I

METALDEHYDE LEVELS IN RAT TISSUE

N.D. = Not detected.

Rat No.	*Dose (mg/kg)*	*Time to death (h)*	*Metaldehyde level found (mg/kg*)*		
			Kidney	*Liver*	*Stomach contents*
1	0	2	16	6	N.D.
2	0	2	0.5	2	N.D.
3	100	2	30	44	924
4	100	2	50	30	1444
5	100	6	42	23	35
6	100	6	14	11	54
7	100	24	13	2	12
8	100	24	9	2	3
9	700	2	64	55	10,270
10	700	1.83	100	55	8100

* Not corrected for background contribution.

Results obtained from the dosed rats are given in Table I. The figures for stomach contents from rats dosed sub-lethally at 100 mg/kg show high concentrations of metaldehyde were present 2 h after dosing but had fallen rapidly after 6 h and approached the normal baseline level after 24 h. Similar changes were found for liver and kidney tissue although they do not show the dramatic rise and fall found for stomach contents. The rats Nos. 9 and 10, administered the LD_{50} dose of 700 mg/kg showed the expected high metaldehyde levels in stomach content. Compared to stomach content levels for rats Nos. 3 and 4, the results for the rats Nos. 9 and 10, administered a potentially lethal dose, reflect the seven times higher dose level. The kidney and liver levels found for rats Nos. 9 and 10 were also significantly higher than the control rats. However it is interesting to note that the metaldehyde levels in liver and kidney of rats Nos. 9 and 10 did not increase by a similar factor as shown by stomach contents compared to rats Nos. 3 and 4. The identical levels found for liver tissue in rats Nos. 9 and 10 may indicate a saturation level has been reached. The gas chromatogram obtained from the head-space analysis of liver tissue from the sub-lethally dosed rat No. 4 is shown in Fig. 5.

The chromatogram obtained by head-space analysis of acidified kidney tissue from a hedgehog thought to have died from metaldehyde poisoning is shown in Fig. 6. The acetaldehyde peak is equivalent to a level of 60 ppm metaldehyde, clearly sufficient to have caused death.

The results obtained here demonstrate the usefulness of the method for the

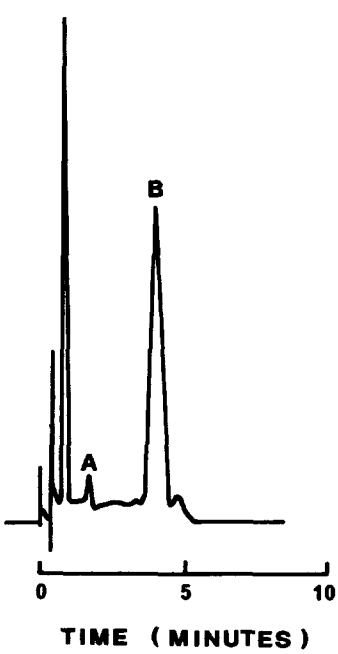

Fig. 4. Gas chromatogram of head-space above acidified liver from control rat No. 2. Peak A and B and GC conditions as in fig. 1.

Fig. 5. Gas chromatogram of head-space above acidified liver tissue taken from rat No. 4, dosed at 100 mg/kg. Peaks A and B and GC conditions as in fig. 1.

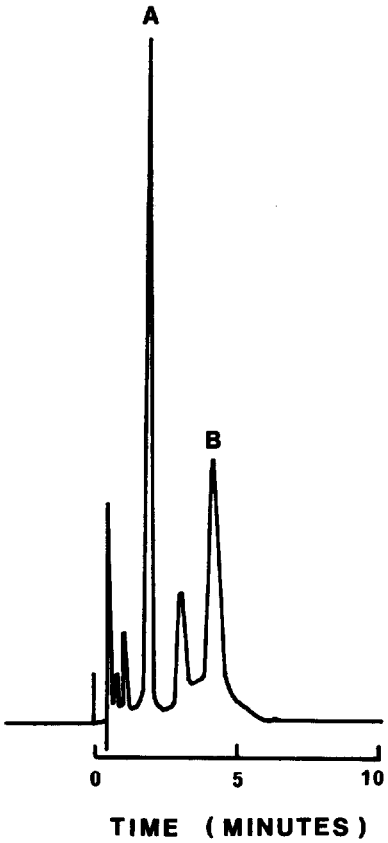

Fig. 6. Gas chromatogram of head-space above acidified kidney tissue taken from hedgehog. Peaks A and B and GC conditions as in fig. 1.

determination of metaldehyde in biological tissue. Furthermore they show that analysis of stomach contents, liver and kidney can provide evidence of metaldehyde ingestion. Stomach contents are obviously the best material to use for detection of metaldehyde poisoning because of the very low background of acetaldehyde. However, levels of 50–60 mg/kg or higher in liver and kidney tissue are sufficient proof of death through metaldehyde poisoning. These results (Table I) refer to studies on rats but they should provide a useful guide for canine poisoning since the lethal dose for dogs is approximately the same, (dog, oral LD_{50} = 600 mg/kg)[11].

CONCLUSION

The head-space method described here for the determination of metaldehyde in biological tissue has several advantages over the spot test method previously employed, in that the method is quantitative, more sensitive and reliable. In addition the method is quick and easy and requires readily available apparatus.

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REFERENCES

1 N. G. Hadden, *Garden Illus.*, 58 (1936) 255.
2 J. R. Cutler, *Survey Rept. No. 27 Review of Pesticide Usage in Agriculture, Horticulture and Animal Husbandry, 1975–1979*, Agricultural Scientific Services, Edinburgh, 1981, p. 12.
3 T. Turner, *Vet. Rec.*, 74 (1962) 592.
4 M. N. Egyed and Y. L. Brisk, *Vet. Rec.*, 78 (1966) 753.
5 S. Selim and J. N. Seiber, *J. Agric. Food Chem.*, 21 (1973) 430.
6 Y. Kimura and V. L. Miller, *J. Agric. Food Chem.*, 12 (1964) 249.
7 D. L. Mays, G. S. Born and J. E. Christian, *Bull. Environ. Contam. Toxicol.*, 3 (1968) 366.
8 E. G. C. Clarke, *Poisoning in Veterinary Practice*, The Association of the British Pharmaceutical Industry, London, 1975, p. 17.
9 G. L. Wardall and S. Bailey, *Vet. Rec.*, 99 (1976) 274.
10 F. Feigl, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 5th ed., 1956, p. 334.
11 E. J. Fairchild, *Agricultural Chemicals and Pesticides–A Handbook of the Toxic Effects*, Castle House Publications, Tunbridge Wells, 1978.