New inhibitors of methane production by rumen micro-organisms. Experiments with animals and other practical possibilities

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1. Two inhibitors of rumen methane production were given to sheep and their effects were measured using an in vitro technique.

2. The substances used were trichloroethyl pivalate (TCE-P) and trichloroethyl adipate (TCE-A). In one experiment with two sheep TCE-P was injected into the rumen and in another experiment with two sheep the same inhibitor was mixed with the food just before feeding. In another experiment, TCE-A was given to two sheep at two dose levels, and in an experiment with four sheep two levels of inhibitor and two levels of diet were used.

3. In general the inhibition of methane production was greater with samples of rumen contents taken after feeding than with those taken before feeding. When sheep were given 120–300 mg TCE-P/d the inhibition of methane production ranged from 21 to 81 %. When sheep given maintenance rations were given 125–300 mg TCE-A/d there was 28–90 % inhibition of methane production. When sheep were given twice maintenance rations 150 mg TCE-A/d gave no inhibition and 300 mg TCE-A/d gave very low inhibition of methane production (about 16 %).

4. In most experiments there was a significant increase in the propionic acid:acetic acid concentration ratio in the rumen when methane production was inhibited.

5. The possible practical use of inhibitors is discussed.

Several attempts have been made to inhibit methane production in ruminants by administering polyhalogenated compounds to the animals. A number of compounds have been used, the simplest being the trichloro analogue of methane, chloroform. This compound is volatile and cannot be incorporated into food, but its infusion into the rumen of sheep resulted in marked inhibition of methane production (Clapperton & Czerkawski, 1972; Clapperton, 1974).

Trichloroacetaldehyde (chloral) or its hemi-acetal compound with starch have been used extensively. Johnson (1972) found that in sheep about 2 g hemi-acetal/d gave 50–80 % inhibition of methane production. The results of further work (Johnson, 1974) suggested some adaptational responses in nitrogen and energy balance during administration of the hemi-acetal of chloral and starch to sheep. Johnson, Wood, Store & Morgan (1972) found that bromochloromethane increases the average daily body-weight gain of steers given 5·5 g/d.

Sawyer, Hoover & Sniffen (1974) administered two levels of bromochloromethane to sheep and, apart from inhibition of methane production (85 %), they found that there was an increase in the digestibility of dry matter (DM) and of N. There was an increase also in gross energy and a significant increase in metabolizable energy. The inhibitor was mixed with maize oil and then with pellets, but had to be kept at $-5°$ to prevent evaporation. Bromochloromethane was also used by Trei & Olsen (1969) in sheep and by Trei, Singh & Scott (1970) in steers.

Chloral hydrate was given to animals by Prins (1965) and by Quaghebeur & Oyaert (1971) and although methane production was not measured, the changes in fermentation patterns were consistent with inhibition of methane production. Trei, Parish, Singh & Scott (1971) tested halogenated compounds in vitro and trichloroacetamide in an experiment with lambs and found that there was an improved food conversion ratio.

In the experiments described here no attempt was made to study the animal's performance. The object was to give some of the inhibitors developed in this laboratory (Czerkawski & Breckenridge, 1975) to sheep, if possible over a long period of time, and to measure the effect on methane production and fermentation patterns in the rumen. It was also of interest to determine whether the inhibitors would reduce the food consumption, whether there was any adaptation and whether the inhibitors could be used under practical conditions.

The composition of the microbial population in strained rumen contents is not necessarily representative of the whole microbial population in the rumen. However, the use of semi-solid digesta for incubations was impracticable because of heterogeneity of samples to be incubated concurrently and because it would preclude the simple use of syringes as combined reaction vessels and collectors of gas. During the development of inhibitors the assays were of comparative nature and it seemed sufficient that the population of strained rumen contents was related to the whole bacterial population. The limitations of strained rumen contents also applied during assays of inhibitory activity in the rumen, but again it would not be practicable to use semi-solid digesta. The compounds tested were only sparingly soluble in water and tended to associate with solid particles. Therefore, the use of strained rumen contents would tend to underestimate the extent of inhibition.

Two potential inhibitors were selected for the present experiments, trichloroethyl pivalate (TCE-P) and trichloroethyl adipate (TCE-A). There was some evidence that both compounds were active in their ester form, although on hydrolysis both gave at least one fragment (trichloroethanol) that was active itself. Water-soluble inhibitors did not seem desirable for practical purposes since their concentration after feeding might assume harmful proportions and, because of rumen flow, they would be subsequently washed out and lost. The inhibitors used here are only sparingly soluble in water and as they are 'lipid-like', they are likely to associate with the solid particles and therefore remain in the rumen longer than the soluble inhibitors. Furthermore, because of the 'lipid-like' properties of the inhibitors, it seemed possible that they might be suitable for application on pasture; it was, in fact, shown that this procedure might be quite feasible.

EXPERIMENTAL

Methane production and fermentation patterns

The methane production was determined by the 'zero-time' technique, in which it is assumed that samples of rumen contents incubated under suitable conditions will for a short time behave as they would do in the rumen. Samples of rumen contents were strained through gauze and incubated in glass syringes as described by Czerkawski &

Breckenridge (1975), except that no inhibitor was added and the samples were incubated for 3 h. At the end of incubation the gases were analysed as described by Czerkawski & Clapperton (1968).

In some experiments the changes in fermentation pattern were studied by determining the concentration of volatile fatty acids (VFA) in the rumen. These were determined by gas–liquid chromatography according to the method of Cottyn & Boucque (1968). The inhibitors used in these experiments, TCE-P and TCE-A, were prepared as described by Czerkawski & Breckenridge (1975).

Animals and diets

The sheep used were 2–4-year-old wethers with rumen fistulas. Two types of concentrate diet were used, molassed sugar-beet pulp and goat mix, and both diets were supplemented with roughage in the form of hay. The goat mix, rolled oats–decorticated-cottonseed cake–bean meal–cooked, flaked maize (22:7:7:4, by wt), was obtained from Paterson Connell, Kilmarnock. It also contained 9 g dairy cattle mineral mixture/kg.

Experiments with TCE-P

Two experiments were done in this series. In the first exploratory experiment two sheep given different rations were used. One sheep was given 300 g goat mix at 09.00 hours and 700 g hay at 16.00 hours and the other sheep was given 500 g molassed sugar-beet pulp at 09.00 hours and 500 g hay at 16.00 hours. After about 10 d on the ration, samples of rumen contents were taken 4 h after the morning feed for 3 d and incubated to determine the control methane production. At this stage, the inhibitor TCE-P, 100 μl in 100 ml Tween 80 (Honeywill & Stein Ltd, London W1) solution (5 g/l), was injected into the rumen of the sheep during the morning feed. This was followed by another control period of 5 d which in turn was followed by periods of 10 and 6 d during which the inhibitor (100 and 200 μl TCE-P respectively) was added in *n*-hexane to the daily concentrate ration. The inhibitor was dissolved in 10 ml *n*-hexane and shaken with the ration in Polythene bags; the hexane was allowed to evaporate for 1 h and the rations were then given to the sheep.

In the second experiment with TCE-P, two sheep were used. The basal ration consisted of 500 g goat mix given at 09.00 hours and 500 g hay given at 16.00 hours. The inhibitor (250 μl (300 mg) TCE-P) was injected with a microsyringe into the morning rations of sheep about 10 min before the morning feed, the food was shaken and given to the sheep. The inhibitor was added during weeks 4, 5 and 6 with sheep 434 and weeks 2, 3 and 4 with sheep 475. Samples of rumen contents were taken before and 2 h after the morning feed. Thus any inhibition of methane production with the samples taken before the morning meal must have been due to a residual effect of the inhibitor given 24 h earlier.

Experiments with TCE-A

In the first experiment with TCE-A two sheep were used. They were each given 200 g cubes made of goat mix at 09.00 hours and 600 g hay at 16.00 hours. The control period was 3 weeks and it was followed by two periods of 3 weeks in which the

sheep were given 200 g goat mix containing 125 and 250 mg TCE-A respectively. Samples of rumen contents were taken before and 2 h after the morning meal on the final 2 d of the 2nd and 3rd week in each treatment and incubated to determine methane production. The concentrations of VFA were also determined.

In the second experiment with TCE-A, four sheep were used. There were six treatments each given for periods of 3 weeks. Each sheep was given 200 g hay with the meal at 16.00 hours. The concentrate, goat mix, was given at two levels, 600 and 1200 g/d in two equal meals at 09.00 and 16.00 hours. All the sheep were kept on the low-level (600 g goat mix/d) control ration for 3 weeks before the start of the treatments. The treatments were: *A*, *B*, *C*, 600 g goat mix/d with 0, 0·25 or 0·5 g TCE-A/kg respectively; *D*, *E*, *F*, 1200 g goat mix/d with 0, 0·125 or 0·25 g TCE-A/kg respectively. The treatment sequences for sheep 552, 557, 561 and 563 were *ABDFEC*, *BCEAFD*, *CDFBAE* and *DEACBF* respectively. The experimental design used was a partially balanced change-over with six treatments, six periods and four animals in a single block. Methods of constructing a design of this type and analysing the resulting data are described by Patterson & Lucas (1962). The randomization of the treatment sequences within periods allowed the presence of residual effects to be tested, although with rather low efficiency.

The sheep were weighed before the morning meal just before the change of rations. Samples of rumen contents were taken before and 2 h after the morning meal on the final 2 d of the 2nd and 3rd week of each treatment. The samples of rumen contents were analysed for VFA and were incubated to determine methane production.

The third experiment with TCE-A was similar to the first experiment with the same inhibitor. Two sheep were given goat mix (200 g) and hay (600 g) at 09.00 and 16.00 hours respectively. During weeks 1, 2 and 3 the sheep received control rations, during weeks 4, 5, 6, 7 and 8 they received the goat mix containing TCE-A (1·5 mg/g) and during weeks 9 and 10 the sheep were again given the control diet. Samples of rumen contents were taken before and 2 h after the morning meal twice per week (on consecutive days) and the methane production was determined.

Application of TCE-A on pasture

In the first three experiments, four plots, 0·84 m^2, were marked out on ryegrass leaving at least 0·3 m strips between the plots. The grass was cut to about 10 mm in length, allowed to grow for a few days and then sprayed with the inhibitor in *n*-hexane solution. Two plots had no treatment, 200 ml *n*-hexane was sprayed on another plot and 100 mg TCE-A in 200 ml *n*-hexane was sprayed on the fourth plot. After a suitable time interval, an area of grass 0·093 m^2 (1 ft^2) was cut as close to the ground as possible from the region of one corner of each plot (about 150 mm from the edge). The samples were weighed and 40 g of each was washed with *n*-hexane as follows. The grass was suspended in *n*-hexane (200 ml) for 1 h, decanted and washed twice with 100 ml *n*-hexane. The three extracts were pooled, evaporated to dryness using a rotary evaporator and weighed. The extracted grass was dried at 105° to determine the DM content.

The extract residues from 40 g grass were emulsified with 10 ml Tween 80 solution

Table 1. *Effect of trichloroethyl pivalate (TCE-P) on the production of methane and carbon dioxide (ml/3 h) by rumen contents from sheep 493 given 300 g goat mix* and 700 g hay/d and from sheep 361 given 500 g molassed sugar-beet pulp and 500 g hay/d*

(Mean values and standard deviations; no. of incubations of samples taken on different days in parentheses)

	Sheep 493				Sheep 361			
	Methane		Carbon dioxide		Methane		Carbon dioxide	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control (no TCE-P) (2)	0·82	0·42	4·05	1·60	2·60	0·40	6·20	1·50
TCE-P 120 mg† (2)	0·38	0·09	4·20	0·60	nd		nd	
Control (3)	0·80	0·07	4·33	0·40	1·69	0·36	6·01	0·55
TCE-P 120 mg‡ (6)	0·64	0·18	3·85	0·78	1·16	0·07	4·57	0·16
TCE-P 240 mg‡ (4)	0·38	0·17	3·87	0·84	1·06	0·18	8·18	0·39

nd, Not determined.

* Rolled oats–decorticated-cottonseed cake–bean meal–cooked flaked maize (22:7:7:4, by wt) and 9 g dairy cattle mineral mixture/kg mix.

† Injected into the rumen.

‡ Incorporated into feed.

(5 g/l) and 1 ml portions were incubated with rumen contents to test for inhibitory activity.

In the final experiment, again four plots of grass were marked out and prepared as before but this time increasing amounts of TCE-A were used (0, 120, 240 and 480 mg TCE-A in 100 ml *n*-hexane/m^2). Small samples of grass (areas 150 × 50 mm) were cut 1 h after spraying and for up to 9 d after spraying. The samples from each plot were taken in a random manner. These samples (20–30 g) were placed immediately after cutting into flasks containing 100 ml *n*-hexane. After two further extractions with 100 ml *n*-hexane the residues of the evaporated extracts were emulsified and tested for inhibitory activity as before.

RESULTS

Experiments with TCE-P

The results of the first experiment with TCE-P are shown in Table 1. With the sheep given goat mix the inhibition of methane production increased with the amount of inhibitor introduced and it appeared that the inhibitor was more effective when it was introduced directly into the rumen. There was no marked decrease in the production of carbon dioxide during the incubations, suggesting that the rest of the fermentation was not affected.

In the second experiment with TCE-P, the samples taken before the morning feed showed little inhibition of methane production (Table 2). There was marked inhibition of methane production during incubation of samples taken 2 h after the morning meal and there was a significant increase in the accumulation of hydrogen. There was no indication that the inhibition was less at the end of the 3rd week rather than at the end

Table 2. *Effect of trichoroethyl pivalate (TCE-P) on the production of methane (ml/3 h) and hydrogen (μl/3 h) by rumen contents from two sheep (nos. 434 and 475) given goat mix* and hay (500 g of each/d)*

(Mean values for single determinations on 2 consecutive days)

Time after start of experiment (weeks)	Sheep 434					Sheep 475				
		Methane		Hydrogen			Methane		Hydrogen	
	Treatment	B	A	B	A	Treatment	B	A	B	A
2	C	1·52	1·17	45	50	I	—	—	—	—
3	C	1·25	1·22	35	80	I	1·39	0·28	40	240
4	I	—	—	—	—	I	0·66	0·13	80	280
5	I	0·85	0·77	80	300	C	—	—	—	—
6	I	1·10	0·04	27	460	C	1·29	1·65	10	20
7	C	—	—	—	—	C	2·20	2·12	8	10
8	C	1·73	1·83	9	15	—	—	—	—	—

C, control (no inhibitor); I, 300 mg TCE-P added to goat mix just before feeding; B, samples of rumen contents taken before morning meal; A, samples taken 2 h after morning meal.

* Rolled oats–decorticated-cottonseed cake–bean meal–cooked flaked maize (22:7:7:4, by wt) and 9 g dairy cattle mineral mixture/kg mix.

Table 3. *Effect of trichloroethyl adipate (TCE-A) on the production of methane (ml/3 h) and hydrogen (μl/3 h) by rumen contents from two sheep given 200 g goat mix and 600 g hay/d*

(Mean values and standard deviations for one determination/sheep per d, made on the final 2 d of the 2nd and 3rd week of each treatment)

Treatment	Methane				Hydrogen				Ratio, propionic acid:acetic acid	
	B		A		B		A			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	B	A
Control	2·31	0·13	2·45	0·15	18	3	11	10	0·25	0·28
TCE-A 125 mg	1·93	0·09	0·44	0·29	6	15	212	35	0·36	0·33
TCE-A 250 mg	0·48	0·12	0·05	0·06	227	57	400	43	0·50	0·54

B, samples of rumen contents taken before morning meal; A, samples taken 2 h after morning meal.

* Rolled oats–decorticated-cottonseed cake–bean meal–cooked flaked maize (22:7:7:4, by wt) and 9 g dairy cattle mineral mixture/kg mix.

of the 2nd week of administration of the inhibitor. When methane production was inhibited, the propionic acid:acetic acid ratio changed in favour of propionic acid in sheep 434 but not in sheep 475. In sheep 434 the mean values for the ratio were 0·24 and 0·27 before and after feeding control rations respectively and 0·33 and 0·54 before and after feeding rations containing TCE-P respectively.

Experiments with TCE-A

The incorporation of TCE-A in diets resulted in an inhibition of methane production (Table 3). The extent of inhibition was greater with samples taken 2 h after feeding and increased with increasing amounts of inhibitor. Again, the inhibition was

Table 4. *Effect of trichloroethyl adipate (TCE-A) on the production of methane by rumen contents from sheep given two levels of TCE-A at two levels of feeding**

(Mean values for four sheep. Samples of rumen contents taken before and 2 h after the morning meal on the final 2 d of the 2nd and 3rd week of each treatment)

Treatment	Level of feeding	TCE-A (mg/d)	Methane production (ml/3 h)			
			Before feeding		2 h after feeding	
			Week 2	Week 3	Week 2	Week 3
A	Low	0	3·53	2·45	4·47	3·03
B	Low	150	2·76	1·54	2·81	2·43
C	Low	300	2·76	2·22	2·22	2·03
D	High	0	2·75	3·42	3·85	3·48
E	High	150	3·05	3·78	3·49	3·88
F	High	300	3·23	2·50	2·97	3·21

* 200 g hay and 600 (low) or 1200 (high) g goat mix (rolled oats–decorticated-cottonseed cake–bean meal–cooked flaked maize (22:7:7:4, by wt) and 9 g dairy cattle mineral mixture/kg mix)/d.

associated with accumulation of hydrogen and with an increased propionic acid:acetic acid ratio.

The results of the second experiment were not as conclusive as those of the first experiment with TCE-A (Table 4). The sheep used in this experiment were relatively large (mean body-weight (kg): at the start of the experiment 50, at the end of the experiment 63), the level of feeding was higher than in the other experiments and the inhibitor was given twice daily.

In this experiment there were six treatments, but only four sheep were used, therefore the design was not the usual Latin-square type. Statistical analysis showed that according to the over-all treatment effects (adjusted for residual effects) the differences were not significant. However, the individual comparisons suggested that the differences between some of the mean values could be real. This view was strengthened by the results of other experiments with TCE-A. In general, the inhibition of methane production appeared to be greater during incubation of post-feeding samples. The inhibition was more evident at low levels of feeding (600 g goat mix/d) but not at high levels (1200 g goat mix/d), even with the greater concentration of inhibitor. The results obtained after 2 weeks on each ration were not different from those obtained after 3 weeks on the ration.

Some of the results of the third experiment with TCE-A are given in Fig. 1. In this experiment the sheep were given TCE-A for 5 weeks (weeks 4–8 of experiment). Clearly, during weeks 4 and 5 of the experiment there was marked inhibition of methane production with samples of rumen contents taken both before and 2 h after feeding. The rumen began to adapt and during weeks 7 and 8 of the experiment methane production with prefeeding samples was the same as the control methane production. Inhibition of methane production persisted in post-feeding samples throughout the feeding of TCE-A, although here too, there was some evidence of adaptation. These changes were accompanied by accumulation of hydrogen gas and an increase in the propionic acid:acetic acid ratio, but the results are not given here.

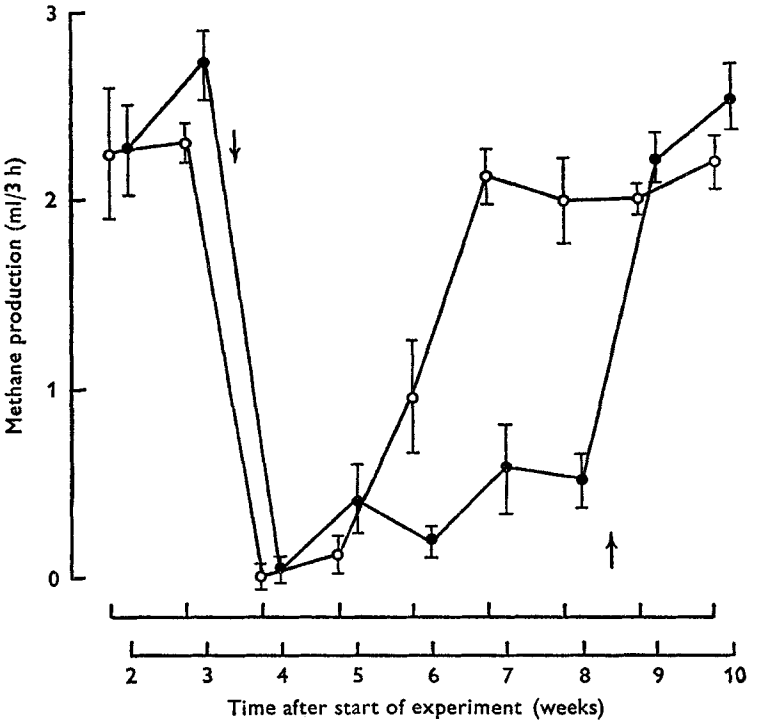

Fig. 1. Production of methane by rumen contents from sheep given control rations (600 g hay and 200 g goat mix/d) (weeks 1, 2, 3, 9 and 10 of experiment) and control rations containing 1·5 g trichloroethyl adipate/g goat mix (300 mg/d) (weeks 4–8 of experiment; indicated by arrows). Each point represents the mean value for results of single incubations, on 2 consecutive days, of samples of rumen contents taken from two sheep, i.e. mean values for four incubations. ○—○, Samples taken before feeding; ●—●, samples taken 2 h after feeding; vertical bars represent twice the standard error of the mean. For details of composition of goat mix, see p. 449 and for details of experiment, see pp. 449–50.

Application of TCE-A on pasture

In the preliminary experiment done during spring, four plots were used, of which two were not treated, one plot was sprayed with *n*-hexane and one plot was sprayed with 120 mg TCE-A in *n*-hexane/m^2. The methane production by rumen contents incubated with extracts of grass untreated with inhibitor was the same as that of the control. The extracts from the treated grass gave consistently 20–30% inhibition of methane production by rumen contents. Czerkawski & Breckenridge (1975) found that the amount of TCE-A which gives 50% inhibition is 52 μmol/l rumen contents, i.e. 0·56 mg/incubation vessel and therefore one would expect 25% inhibition with 0·28 mg TCE-A. In 0·84 m^2 area of grass there is about 1·5 kg fresh material of which 40 g was used for extraction. Thus we would expect $100 \times 40/1500 = 2{\cdot}6$ mg TCE-A/extract and 0·26 mg/incubation; this value agrees with the determined value.

However, in two other experiments, when the samples of grass were taken for up to 18 d, the extracts had apparently no inhibitory activity, as shown by the methane production during incubation of the extracts with rumen contents (Czerkawski, unpublished results). This could have been due to several causes. There was unusually

Table 5. *Assay of persistence of trichloroethyl adipate (TCE-A) after spraying ryegrass plots with solutions of TCE-A in* n-*hexane*

(The amounts of inhibitor in grass samples were estimated by extracting the grass with *n*-hexane, incubating the extracts with rumen contents and measuring the inhibition of methane production. The recovery was calculated by comparing the inhibition of methane production given by 1 ml ryegrass extracts with inhibition produced by a known amount of TCE-A)

	Methane production (ml/4 h)					Recovery of TCE-A (%)				
Time after spraying (d) ...		2	3	4	6	1	2	3	4	6
Incubation mixture										
Control	2·36	2·36	2·34	2·41	2·70	—	—	—	—	—
TCE-A (0·2 mg/incubation vessel)	1·32	1·32	0·88	1·18	1·35	—	—	—	—	—
Ryegrass extracts										
Hexane	2·52	2·29	2·38	2·57	2·65	—	—	—	—	—
TCE-A 120 mg/m^2	1·48	2·37	1·75	2·63	2·55	69	0	30	0	7
TCE-A 240 mg/m^2	1·22	1·75	1·26	1·06	2·63	43	20	28	42	0
TCE-A 480 mg/m^2	0·64	0·71	0·55	0·70	2·47	31	28	23	25	3

high rainfall between spraying and harvesting and the inhibitor might have been washed out. The experiments were done during late summer, whereas the successful preliminary experiment was done in early spring, therefore the cuticular wax in grass might have been different. Finally, because of unavoidable circumstances, it was necessary to store the harvested grass samples for some days before extraction; the inhibitor might have been hydrolysed by esterases released from the grass.

The results of the last experiment in this series are given in Table 5. Incubations of rumen contents with extracts of grass that was sprayed with *n*-hexane showed that these extracts had no effect on methane production (see Table 5). Comparison of the inhibitory activity of 1 ml grass extract with the activity of known amounts of TCE-A made it possible to calculate the amount of inhibitor per m^2 and hence the recovery. In general, the extent of inhibition increased and the recovery of TCE-A decreased with the amount of inhibitor sprayed. The results were variable but showed that considerable proportions of inhibitor remained on the grass for 4 d after spraying and with the highest rate of application more than 10% could be recovered on day 9 (not shown in Table 5). Throughout the experiment there was considerable rainfall.

DISCUSSION

The extent of inhibition of methane production was calculated by subtracting the value for the methane production by samples taken during control periods and dividing by the control value (Table 6). Although both inhibitors were active, it would be difficult to make definite recommendations about the amounts to be used. A dose of between 200 and 500 mg/d should give good inhibition of methane production for sheep but it would ultimately depend on the size of the animals, the level of feeding, the frequency of dosing and possibly the type of ration.

As expected, the extent of inhibition was greater shortly after ingestion of inhibitor;

Table 6. *Inhibition of methane production by trichloroethyl pivalate (TCE-P) and trichloroethyl adipate (TCE-A) in experiments with sheep: summary of results*

Ration (g/d)	Inhibitor (mg/d)		Inhibition (%) Before feed‖	Inhibition (%) After feed‖
Goat mix* 500, hay 700	TCE-P‡	120	—	53
SBP 500, hay 700	TCE-P	120	—	21
		240	—	53
Goat mix 300, hay 700	TCE-P	120	—	46
		240	—	57
Goat mix 500, hay 500	TCE-P	300	37	81
Goat mix 200, hay 600	TCE-A	125	16	82
		250	79	98
Goat mix 600, hay 200	TCE-A	150	18	25
		300	12	36
Goat mix 1200†, hay 200	TCE-A	150	−6	6
		300	12	28
Goat mix 200, hay 600	TCE-A§	300	63	87

SBP, molassed sugar-beet pulp.

* Rolled oats–decorticated-cottonseed cake–bean meal–cooked flaked maize (22:7:7:4, by wt) and 9 g dairy cattle mineral mixture/kg mix.

† The rations were given at approximately twice the maintenance level.

‡ Inhibitor emulsion injected into the rumen.

§ Calculated from the mean methane production during 4 control weeks and the 1st 4 weeks with TCE-A treatment.

‖ Samples of rumen contents taken before and 2 h after the morning meal.

the residual effect from giving the inhibitor during the previous day was not great. This is not detrimental, since the rate of methane production is greatest 1-4 h after feeding and therefore inhibition is greatest when it is needed most.

It was shown during incubations in vitro (Czerkawski & Breckenridge, 1975) that TCE-P was a more potent inhibitor than TCE-A, particularly when considered on the basis of the active trichloro grouping. In the present in vivo experiments, TCE-P did not appear to be more active than TCE-A. Considering only the results obtained with samples taken after feeding, with sheep at the maintenance level of nutrition and with the inhibitor incorporated into food, the mean amounts of inhibitor which gave 50% inhibition of methane production were 0·89 mmol for TCE-P and 0·38 mmol for TCE-A. Since TCE-A contains two active groups, on the basis of the active groups the amounts of TCE-P and TCE-A which gave 50% inhibition were similar (0·89 and 0·76 mmol respectively).

The pivalate ester was volatile and more soluble than the solid adipate ester. This might account for the apparently lower activity in vivo of the pivalate. However, its activity might be improved if it were incorporated in the food in a micro-encapsulated form.

The two inhibitors tested here were not the most potent ones available, but they appeared to be best from the practical point of view. Admittedly, trichloroethanol is more expensive than chloral, but the price would be reduced if the output increased. The acid part of the ester is relatively cheap, at most 3–5% of the total cost, and in the

instance of TCE-A could be reduced further by utilizing a mixture of succinic, glutamic and adipic acids, such as is used for nylon manufacture.

Even though TCE-P and TCE-A inhibit methane production when given to sheep, there might be no consequential improvement in food conversion. Recently, Dr J. L. Clapperton of this Institute conducted a large-scale trial, in which some lambs were given a control diet and some were given a diet containing 300 mg TCE-A/d. The results of this experiment with one inhibitor given at one level, were inconclusive inasmuch as the treatment did not significantly affect the food conversion (J. L. Clapperton, unpublished results). It is possible that the level of TCE-A used was too low or that the rumen micro-organisms became adapated. Another possibility that must be considered is that there might be an improvement in food conversion in the rumen accompanied by a detrimental effect on the metabolism of the host animal.

When the production of methane in sheep was inhibited by adding linseed oil to the rations (Czerkawski, Christie, Breckenridge & Hunter, 1975), there was a marked increase in the utilization of [^{14}C]acetate for the synthesis of microbial lipids in the rumen. In more recent experiments, when methane production in sheep was inhibited by feeding 400 mg TCE-A/d (Czerkawski, unpublished results), there was again a marked increase in the synthesis of microbial lipids in the rumen. It is not known what are the relative energy costs of synthesis of methane and microbial lipid, but economically, there is no doubt that a digestible microbial lipid is better than the eructed methane.

The results of these preliminary experiments, although they were only exploratory, showed that this line of research is promising and might usefully be extended to studies with grazing animals.

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