

# Residue levels in cows after oral ingestion of methylthiouracil

R. VERBEKE, H.F. DE BRABANDER AND A. ERMENS

Laboratory of Chemical Analysis of Food of Animal Origin, Veterinary Faculty,  
University of Ghent, Belgium

## Introduction

Regulations in the EEC prohibit the use of thyreostatics for cattle fattening. These drugs inhibit the thyroid function : the decreased production of thyroid hormones reduces basal metabolism, lowers gastro-intestinal motility and favors extracellular water retention. The use of these substances thus allows a considerable increase in live weight gain, although this results mainly from an increased filling of the gastro intestinal tract and augmented water retention in the slaughter animals.

In contrast with laboratory animals, where the metabolism of thyreostatics was extensively studied (8), data on the excretion and distribution of thiouracils in cows are rather fragmentary. For regulatory control on the abuse of thyreostatics more information is needed on the excretion and residue levels of thyreostatics in cow tissues after prolonged withdrawal periods. This work was therefore done to investigate the elimination rate and excretion of methylthiouracil (MTU = 4(6)-methyl-2-thiouracil) in cows. Since the weight of the thyroid is often used as a simple parameter to detect MTU-positive carcasses, it seemed desirable to examine the thyroxine levels of cows serum during prolonged MTU feeding.

## Materials and Methods

Twenty one Friesian cows (weight 500-700 kg) were housed in a ventilated stable and individually fed a diet containing a mixture of 80% maize-fodder, 20% fresh grass and 1 kg barley-meal daily/100 kg live weight. After a 15 day preliminary period the cows were allotted to two groups. In the first group, the elimination of MTU was studied in plasma, urine, milk, thyroid and muscle after a single oral administration of MTU ( 8 cows). The animals of the group were slaughtered at 3, 5, 8 or 17 days after MTU treatment. In the second group the animals received a daily dose of 5 g MTU during 2 weeks (2 cows), 3 weeks (3 cows) or 4 weeks (8 cows). The cows were slaughtered at different withdrawal periods (8-78 days).

MTU (usually 5 g) was suspended in 100 ml water and sucked into a plastic syringe. A plastic tube was fitted on the syringe and the suspension ejected at the back of the cows' mouth. Blood was sampled at regular intervals from the vena jugularis in vacuum tubes (10 ml) containing heparin. The blood was immediately cooled in ice-water and centrifuged (1500 g) within 2 h period. The plasma was stored in the deep-freeze (-18°C) until analysis. For thyroxine determination in serum, blood was similarly collected in venoject tubes (i.e. containing no heparin). The blood was kept at room temperature during 6 h after which the tubes were centrifuged and the serum aspirated with a pasteur pipette. The serum was then immediately frozen (-18°C) and analyzed for thyroxine within 3 weeks after sampling. Urine was collected from cows in a

plastic bottle which the aid of an inox catheter. A homogeneous sample (10-20 ml) was transferred in a plastic bottle and immediately frozen at  $-18^{\circ}\text{C}$ . Immediately before slaughter, urine and blood was sampled. Immediately after slaughter, the thyroid was dissected and weighed. Samples were then taken from the thyroid and M. diaphragma, packed in plastic bags and chilled at  $-18^{\circ}\text{C}$ .

MTU determinations were performed on 2 ml urine, plasma, milk, 2 g of meat or thyroid tissue using DMTU as internal standard according to the quantitative technique described previously (7). The results are expressed as mg MTU/kg of tissue or fluid (ppm). Thyroxine ( $\text{T}_4$ ) was estimated in serum using a ELISA method (4) with a kit obtained from BOEHRINGER-MANNHEIM (GFR). Creatinine was determined in urine using a picric acid method (1). Since creatinine is used as an index of urinary excretion (10), the MTU content of urine was expressed as mg MTU/g creatinine.

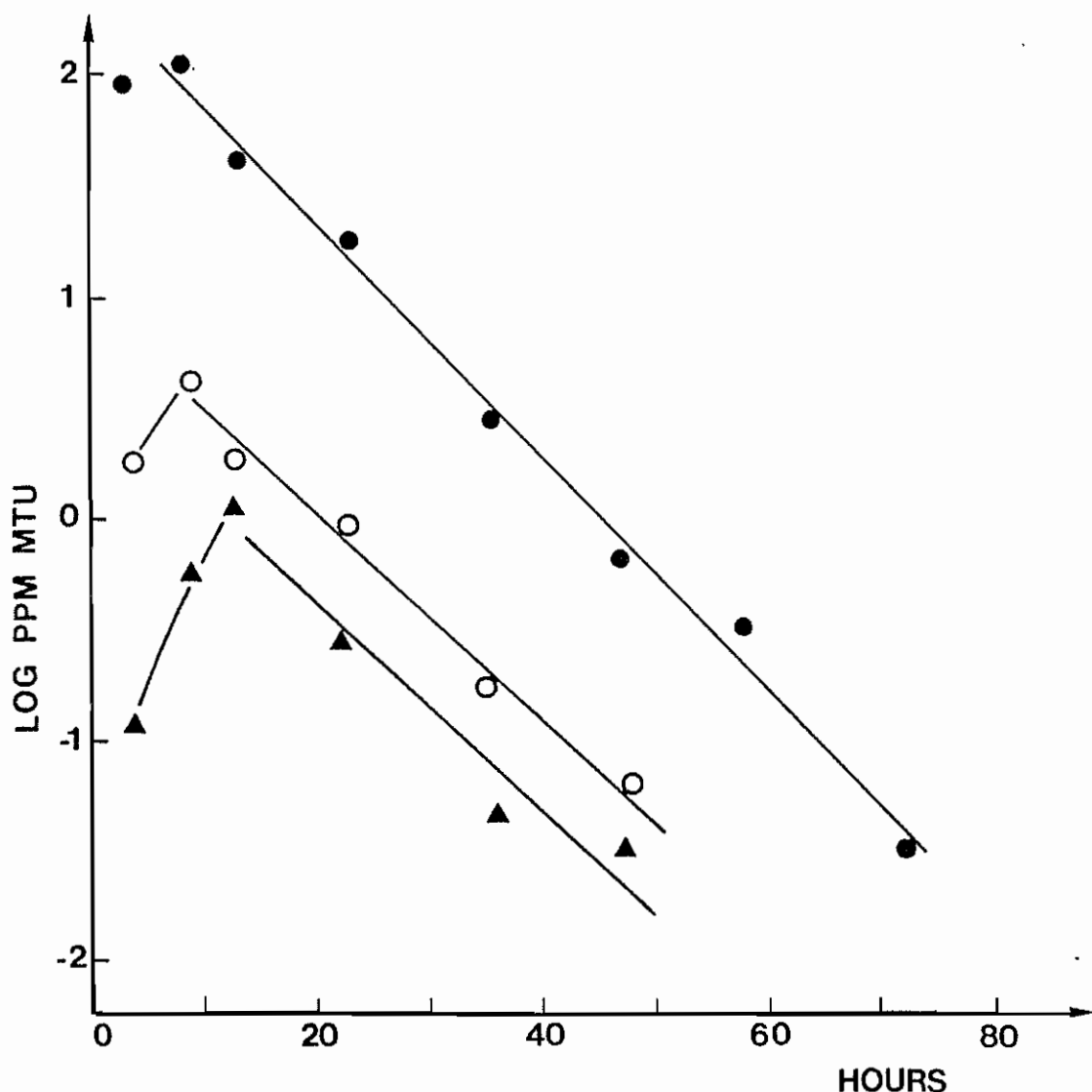


Fig. 1 : MTU levels in plasma (○), urine (●) and milk (▲) at various times after oral administration of 4 g MTU to a cow.

## Results

The results of a representative experiment, obtained after administration of a single oral MTU dose to a cow, are shown in Fig. 1. Maximum MTU levels were found 4-8 h after ingestion in urine, plasma or milk. Concentration of MTU in urine was 50-100 times that of plasma and 250-500 times that of milk. The MTU levels in these fluids show a very rapid and parallel decline in function of time in the first 80 h after treatment. Analysis of plasma or urine, obtained 3-17 days after administration of the drug, show evidence for a second phase during which the elimination of MTU is 15 times slower than observed in the first 80 hours after MTU treatment (Table 1). At slaughter, the MTU concentration in the thyroid was always higher than in plasma or M. diaphragma. Three days after administration of the drug, the residue concentration of MTU in the thyroid was 50-200 times that of M. diaphragma or plasma. The MTU-level in the thyroid decreased in function of the withdrawal period with an apparent half-life of 3 days ( $r = -0.99$ ;  $p \leq 0.001$ ;  $n = 8$  cows).

In the second experiment different animals were treated with a daily dose of 5 g MTU during periods of 2, 3 or 4 weeks. After 10-14 days of MTU treatment the appetite decreased and the reactions of the animals became apathetic. Approximately 5-6 days after stopping the MTU-treatment, there was a temporally increased diuresis, the appetite returned and the reactions of the animals became normal.

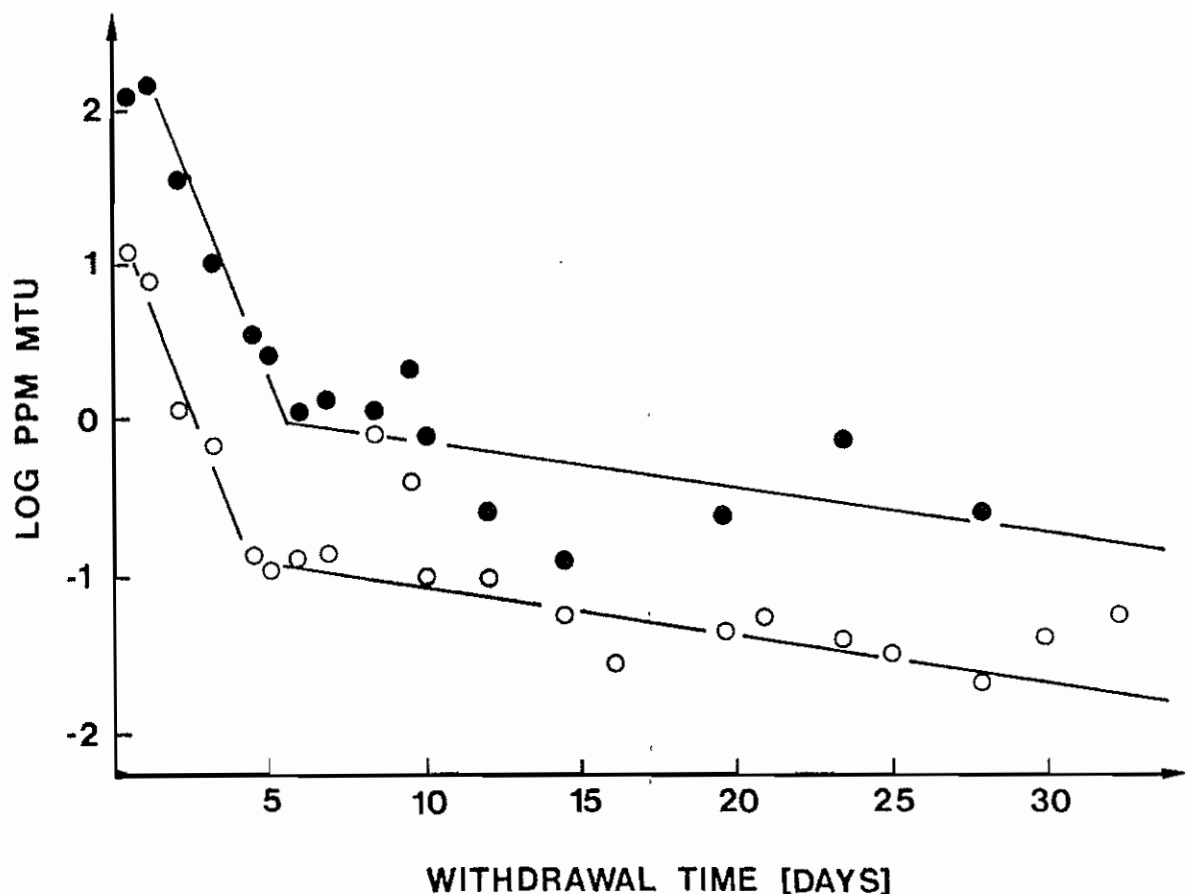


Fig. 2 : Time-course of MTU-levels in urine (●) and plasma (○) in a cow after oral ingestion of a daily dose of 5 g MTU during 21 days.

After a 2, 3 or 4 week MTU-treatment the MTU levels in urine and plasma are 2-3 times higher than the maximum concentration observed after ingestion of a single dose. After withdrawal of the drug, the data show that there are at least 2 phases in the disappearance of MTU from plasma or urine (Fig. 2). In the first 4-5 days after stopping MTU-treatment, the MTU-levels in plasma and urine declined rapidly. Then the elimination rate decreased by a factor 20 so that, after a withdrawal period of more then 60 days, still distinct levels of MTU ( $\geq 10$  ppb MTU) were found in urine or plasma. During that period the residue levels in urine were approximately 5 times higher than in the corresponding plasma samples. There were no significant differences in the elimination constants after feeding MTU to cows during either 2, 3 or 4 weeks. The MTU concentrations in the thyroid were studied over a withdrawal period of 5-80 days (Fig. 3). During this period the apparent half-life of

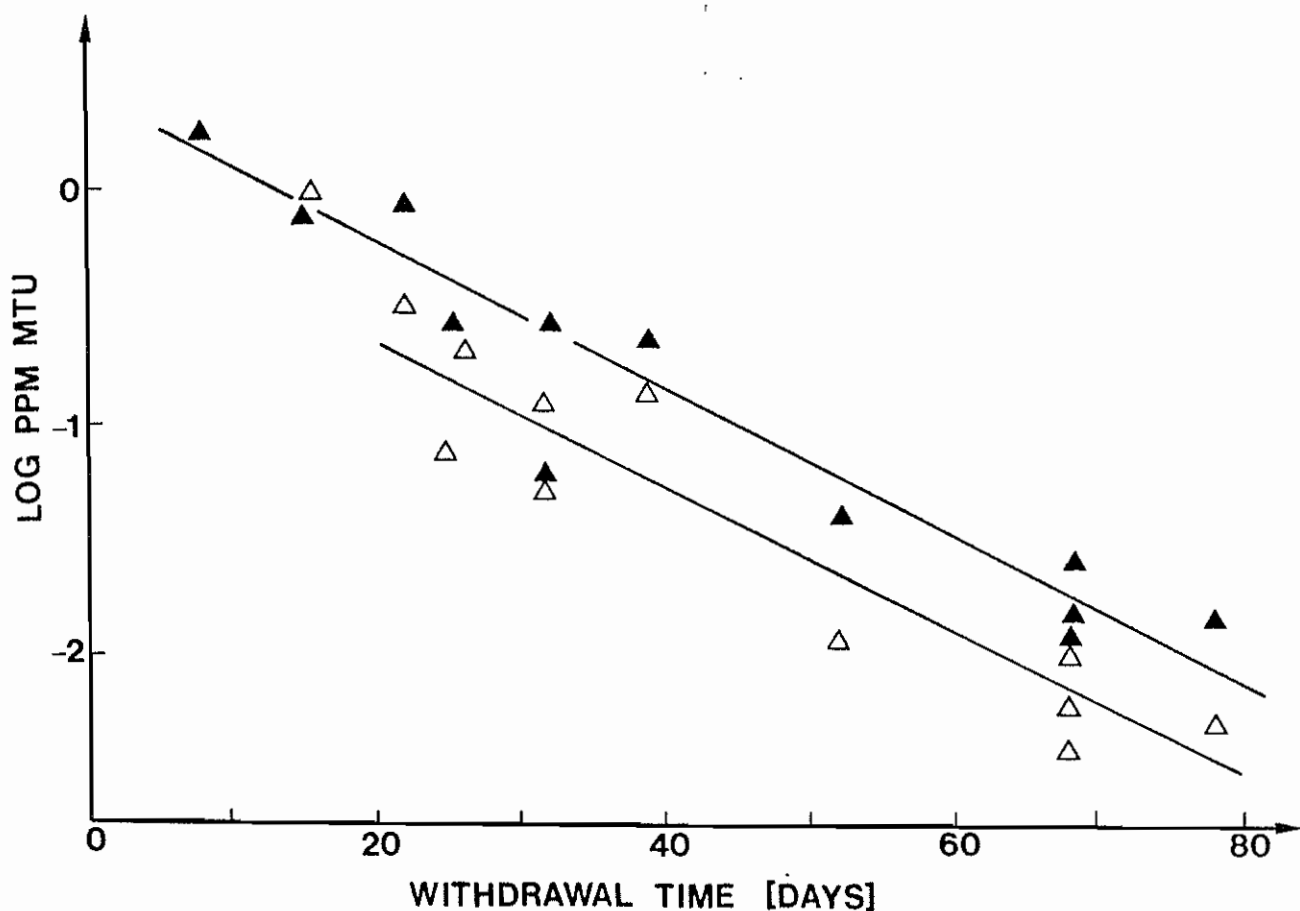


Fig. 3 : Concentration of MTU in the thyroid (▲) and M. diaphragma (△) of cows at various withdrawal periods after daily ingestion of 5 g MTU during 2-4 weeks.

MTU in this organ was at least three times less than that observed in plasma or urine over the same period. In cows, treated with MTU for at least 3 weeks, the MTU concentration in M. diaphragma showed a parallel decrease with that of the thyroid. The MTU residue levels in the thyroid were 3 times higher than that of M. diaphragma over the withdrawal period studied.

The serum levels of thyroxine ( $T_4$ ) were studied in 6 cows during a 3 or 4 week MTU treatment. Normal  $T_4$ -values ranged between 9-20  $\mu\text{g } T_4 / 100 \text{ ml serum}$ . After 10-14 days of MTU treatment the  $T_4$ -values decreased to a minimum 16-22 days after starting the MTU-treatment; the minimum values ranged between 3.2-7.8  $\mu\text{g } T_4 / 100 \text{ ml serum}$ . Similar observations were made by Pohlschmidt & Forschner (12) after feeding MTU to 7 bulls over a period of 26 days; however, their minimal  $T_4$ -values were lower and ranged between 1-2  $\mu\text{g } T_4 / 100 \text{ ml serum}$ . The time-course of the  $T_4$ -values during the experiments were similar if the  $T_4$ -content was expressed as a percentage of its initial value (Fig. 4).: minimal  $T_4$ -values were  $37 \pm 3\%$  of its initial values. Three days after withdrawal of the drug, the  $T_4$  content of serum increased and reached a maximum 5-6 days after stopping the MTU treatment. Then the  $T_4$ -content declined to 70% of its original value after which the  $T_4$ -values started oscillating.

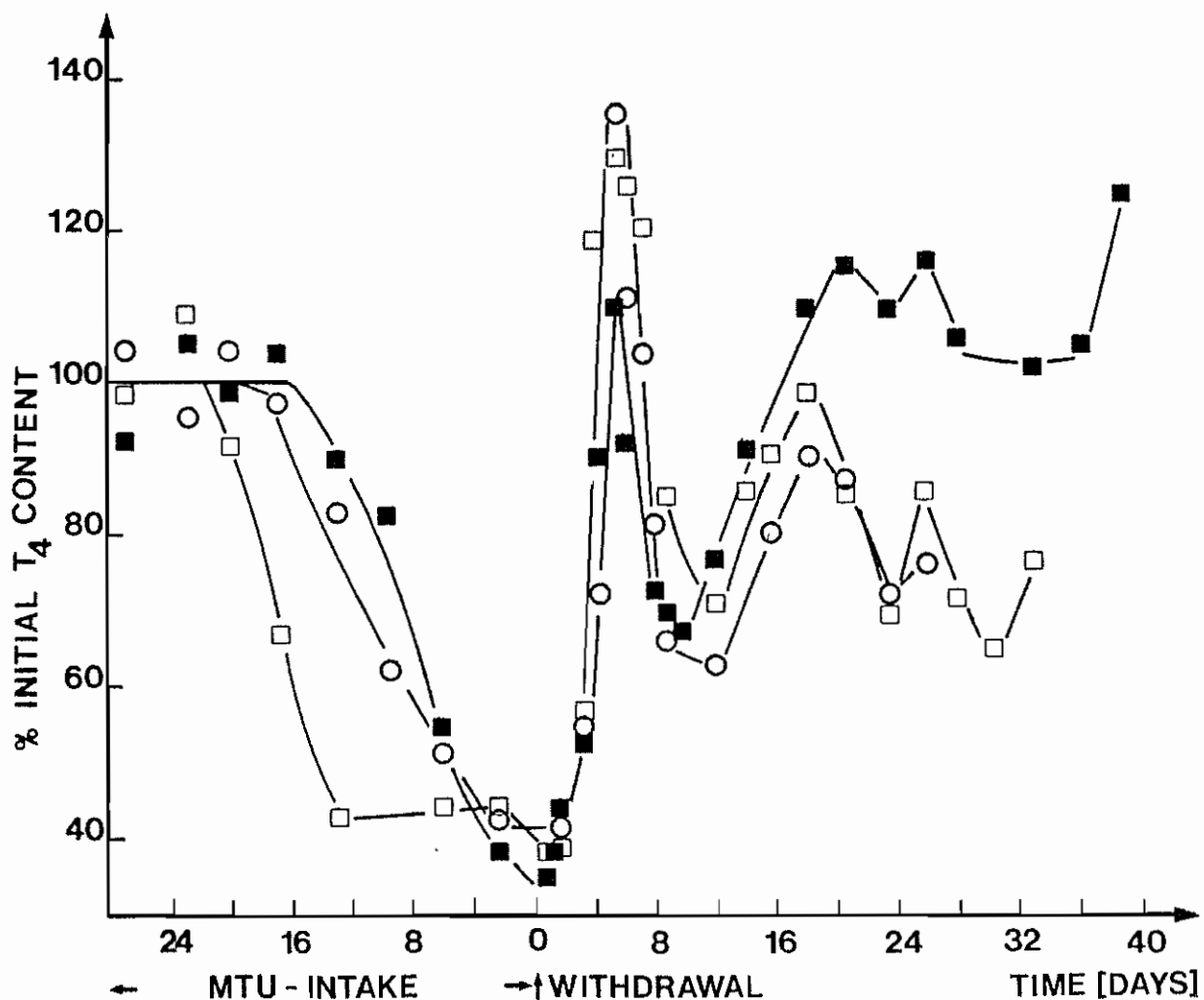


Fig. 4 : Time-course of  $T_4$ -serum levels during and after treatment of 3 cows (■, □, ○) with a daily dose of 5 g MTU ( $T_4$ -values are expressed as percentages of its respective control values).

## Discussion

Data on the excretion and distribution of thiouracils in cows are rather scarce and limited to studies over short withdrawal periods (2, 3, 9). Early studies in ruminants suggested an efficient absorption of the thyreostatic drugs : maximum blood levels were observed 4-5 h after a single oral dose of thiouracil. Elimination was rapid since 24 h later the plasma thiouracil concentration dropped below the 1 ppm level (9). -

Our measurements, obtained after single oral administration of MTU to cows, confirm the initial rapid disappearance of MTU from plasma, urine or milk (Fig. 1). However, 80-100 h after MTU administration, elimination slows down so that 17 days after MTU treatment still appreciable levels of MTU are found in urine and plasma. As shown in earlier experiments (2, 3), the thyroid shows a specific and efficient accumulation of MTU over that of other muscular tissues. After a single oral administration of MTU to cows, the residue ratio of thyroid/M. diaphragma is not constant but drops from 150-200 at 3 days to a value of 1-3 at 17 days after MTU administration.

Table 1 : Half-life of MTU in plasma, urine, muscle and thyroid tissue after oral administration of the drug to cows.

Dose	Fluid or tissue	Half-life of MTU		DT <sup>2</sup>
		first phase	second phase	
single dose				
1 x (2 or 4 g)	urine, milk (2)	6 ± 0.5 h		
1 x 5 g	plasma (8)	6 ± 0.5 h	3.6 days <sup>1</sup>	16 days
1 x 5 g	thyroid (8)	2.9 days		29 days
multiple dose				
14, 21 or 28 x 5 g	plasma,urine (8)	15 ± 3 h	24 ± 1.7 days	65 days
	thyroid (13)	9.7 days <sup>1</sup>		77 days
	M.diaphragma(10)	9.7 days <sup>1</sup>		66 days

( ) : number of cows

<sup>1</sup> : estimated from analysis of samples obtained at slaughter after withdrawal times of at least 3 days.

<sup>2</sup> : withdrawal period (days) during which the concentration of MTU residues in the biological matrix exceeds 0.01 ppm.

After feeding cows with MTU for longer periods (14-28 days) the initial elimination of MTU from plasma, urine or tissues is 2.5-3.3 times slower than observed after a single oral administration of MTU (Table 1). The increased half-life of MTU in urine, plasma or thyroid and the similar half-life of MTU in thyroid and M. diaphragma probably reflects that cow tissues reached steady state levels after feeding MTU for at least 14 days. In comparison with earlier experiments (3, 12), the specific and sensitive detection method employed here allowed detection of significant MTU-levels in the thyroid, muscular tissue, plasma or urine over a tenfold extended withdrawal period.

Prolonged administration of goitrogenic substances to cattle results in symptoms of hypothyroidism: histological hypertrophy of the thyroid gland epithelium or macroscopical enlargement of the thyroid gland (weight) are often used in regulatory control as evidence for illegal treatment with thyreostatics. The time course of the thyroxine levels shows that MTU treatment of cows during one month will not result in a sufficiently long period of hypothyroidism to provoke detectable lesions of the thyroid. This and the inadvertent intake of natural goitrogens via feedstuffs may explain the lack of correlation observed between the results obtained from histological examination and chemical analysis of thyroids taken from regulatory control (13).

From this study it is evident that after MTU-treatment of cows during 4 weeks followed by a withdrawal period of one month still appreciable residue levels (ca 0.1 ppm MTU) may be found in muscular tissue. MTU treatment thus results in higher residue concentrations in muscular tissue during longer withdrawal periods than was expected from earlier experiments (2, 9, 12). For regulatory control on the living animal, urine sampling is the obvious choice. During inspection in the slaughterhouse, thyroid should be selected as the target tissue for optimal detection of the illegal use of thyreostatics.

## Conclusions

Treatment of cows with MTU results in a rapid appearance of the drug in plasma, urine and milk whereas MTU selectively accumulates in the thyroid. MTU levels in plasma or urine show a biphasic elimination: after an initial rapid decline, observed in the first 4 days after treatment, the MTU excretion slows down by a factor 15-30 depending on the treatment. Feeding MTU to cows over prolonged periods results in a slower MTU elimination so that, after a withdrawal period of one month, appreciable residue levels of the drug were found in the thyroid, muscular tissue, urine or plasma.

The time-course of serum thyroxine levels during MTU treatment of cows suggests that histological or macroscopical examination of the thyroid cannot be used as a reliable screening parameter for the illegal use of thyreostatics in cattle fattening. For regulatory control, MTU residues should be monitored in the thyroid, urine or muscular tissue using a sensitive and specific chemical method.

## Aknowledgements

This work was supported by a grant of the Belgian Ministry of Public Health (Meat Inspection). We thank Mrs. M.R. Ruysschaert for expert technical assistance.

## References

1. Bartels, H., Böhmer, M. and Heierte, C. (1972), Clin. Chem. Acta 37, 139-197.
2. Bezem, J.J., Van Esch, G.J. and Peer, H.C. (1949), Landbouwtijdschrift 61, 946-948.
3. Böhnel, T. (1966), Inaugural Diss., Univ. München , G.F.R.
4. Borner, J. Clin. Chem. Biochem. (1979) 17, 474-476.
5. De Brabander, H.F. and Verbeke, R. (1975), J. Chromatogr. 108, 141-151.
6. De Brabander, H.F. and Verbeke, R., (1984), Trends in Analyt. Chem., submitted.
7. De Brabander, H.F. and Verbeke, R. (1984), Proc. 30<sup>th</sup> Eur. Meeting Meat Research Workers, Bristol.
8. Desbarats-Schonbaum, M.L., Endrenyi, L., Kours, E., Schonbaum, E. and Sillers , E.A. (1972), Eur. J. Pharmacol. 19, 104-111.
9. Ely, R.E., Olson, K.J. and Reinecke, E.P. (1947), J. Dairy Sci. 30, 577-581.
10. Erb, R.E., Surve, A.H., Randel, R.D. and Garverick, H.A. (1977), J. Dairy Sci. 60, 1057-1063.
11. Gissel, C. and Schaal, M. (1974), Arch. Lebensmittelhyg. 1, 8-13.
12. Pohlschmidt, J. and Forscher, E. (1979), Fleischwirtsch. 59, 253-255.
13. Pottie, G., (1979), Fleischwirtsch. 59, 248-250.