Elimination profile of methylthiouracil in cows after oral administration[†]

An Heeremans,^{*a*} André Ermens,^{*b*} Katia K. De Wasch,^{*c*} Carlos Van Peteghem^{*a*} and Hubert F. De Brabander^{**c*}

^a Laboratory of Bromatology, Harelbekestraat 72, B-9000 Ghent, Belgium

^b Institute of Veterinary Inspection (IVK), Wetstraat 56, B-1050 Brussels, Belgium ^c Faculty of Veterinary Medicine of the University of Ghent, Department of Veterinary Food Inspection, Laboratory of Chemical Analysis, Salisburylaan 133, B-9820 Merelbeke, Belgium. E-mail: hubert.debrabander@rug.ac.be

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In addition to methods for the determination of residues, there is an important need for knowledge of the fate and excretion of growth promoting substances in fattening animals. In court, often simple questions are asked, such as, over what period of time can the drug be detected?, is it possible to find residues after 2 months, *etc.* These questions can only be answered by conducting animal experiments. Data on excretion and distribution of thiouracils in cows are rather scarce. At the beginning of the 1980s, animal experiments with methylthiouracil (MTU) were carried out in the Laboratory of Chemical Analysis. These experiments showed that treatment of cows with MTU results in the rapid appearance of the drug in plasma, urine and milk, whereas MTU selectively accumulates in the thyroid gland. The results of these experiments were only published in media with limited access (thesis, abstracts) and also there has been a considerable improvement in data handling with computer programs in the last 15 years. This investigation reinterprets the 'old' analytical data from animal experiments using a pharmacokinetic software package.

1 Introduction

Regulations in the EU prohibit the use of thyreostatic drugs for cattle fattening.¹ Thyreostats inhibit the thyroid function: the decreased production of thyroid hormones reduces basal metabolism of the animals, lowers gastro-intestinal motility and favours extracellular water retention. The use of these substances therefore allows a considerable increase in live mass gain, although this mainly results from an increased filling of the gastro-intestinal tract and augmented water retention in the slaughtered animals.

For regulatory control of the abuse of thyreostatics, information is needed on the excretion and residue levels of thyreostatics in tissues after prolonged withdrawal periods. In court, often simple questions are asked, such as, over what period of time can the drug be detected?, is it possible to find residues after 2 months, etc. These questions can only be answered by conducting animal experiments in which certain amounts of the drug are administered and samples of excreta are analysed at regular time intervals. For a number of thyreostats, methods for their detection were worked out and data on their excretion were gathered and published (e.g., the inorganic ions perchlorate, thiocyanate and lithium).2-4 These experiments were not intended as real pharmacological studies as for legal drugs but only to inform the analyst and the inspection services on the nature and the magnitude of the concentrations of drugs which could be expected after illegal treatment of animals.

The metabolism of thiouracils has been extensively studied⁵ in laboratory animals. However, data on the excretion and distribution of these thyreostats in cows are fragmentary. At the beginning of the 1980s, experiments were carried out in the Laboratory of Chemical Analysis in order to investigate the

elimination rate and excretion of methylthiouracil [MTU, 4(6)-methyl-2-thiouracil] in cows. Since the mass of the thyroid is often used as a simple parameter to screen for MTU-positive carcasses, it seemed desirable to examine the thyroxine levels in cows' serum during prolonged MTU feeding.

The results of these experiments were only published in theses^{6,7} or congress proceedings,⁸ which limits the availability of the data. Moreover, in the last 15 years there has been a considerable improvement in data handling with computers. Therefore, this investigation reinterprets the 'old' analytical data from our animal experiments using the MW/Pharm pharmacokinetic software package,⁹ in order to obtain a detailed elimination profile of MTU after oral administration and to bring these data into the open literature. However, in this investigation only a selection of the data could be presented. The complete set of raw and processed data is available on request through the Community Reference Laboratory.

2 Experimental

Friesian cows (500–700 kg) were housed in ventilated stables and individually fed a diet containing a mixture of 80% maizefodder, 20% fresh grass and 1 kg barley-meal daily per 100 kg live mass. 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 (eight cows). Two animals in the group were slaughtered at 3, 5, 8 or 17 d withdrawal period after MTU treatment. In the second group the animals received a daily dose of 5 g of MTU for 2 weeks (two cows), 3 weeks (three cows) or 4 weeks (eight cows). The cows were slaughtered at different withdrawal periods (8–78 d). For this experiment, no control animals were used but excreta of all animals taken before MTU administration were analysed and found to be negative for MTU.

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MTU (usually 5 g) was suspended in 100 ml of water and drawn 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 (1500g) within a 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 for 6 h, after which the tubes were centrifuged and the serum aspirated with a Pasteur pipette. The serum was then immediately frozen $(-18 \,^{\circ}\text{C})$ and analysed for thyroxine within 3 weeks after sampling. Urine was collected from cows in a plastic bottle with the aid of an inox catheter. A homogeneous sample (10-20 ml) was transferred into a plastic bottle and immediately frozen at -18 °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 frozen at -18 °C.

MTU determinations were performed on 2 ml of urine, plasma and milk and 2 g of meat or thyroid tissue using dimethylthiouracil (DMTU) as internal standard according to the quantitative HPTLC technique described previously.^{7,10,11} The results are expressed as mg MTU kg⁻¹ tissue or fluid (ppm). Since these analyses were time consuming, only one analysis per sample was carried out. Thyroxine (T₄) was determined in serum using a ELISA method¹² with a kit obtained from Boehringer Mannheim (Mannheim, Germany). Creatinine was determined in urine using a picric acid method.¹³ Since creatinine is used as an index of urinary excretion,¹⁴ the MTU content of urine was expressed as mg MTU g⁻¹ creatinine.

3 Results and discussion

Elimination of MTU after ingestion of a single dose

Data on the excretion and distribution of thiouracils in cows are scarce and limited to studies over short withdrawal periods.^{15–17} Early studies in ruminants suggested 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 level¹⁷ (which was the limit of detection of the method used at that time).The results of our first experiment, obtained after administration of a single oral MTU dose of 4 g to a cow, are shown in Fig. 1.

Maximum MTU levels were found 4–8 h after ingestion in urine, plasma or milk. The concentration of MTU in urine was 50–100 times that in plasma and 250–500 times that in milk. The MTU levels in these fluids show a very rapid and parallel decline as a function of time in the first 80 h after treatment. Our measurements, obtained after single oral administration of MTU to cows, confirm the initial rapid disappearance of MTU



Fig. 1 MTU levels in plasma, urine and milk after oral administration of 4 g of MTU to a cow.

from plasma, urine or milk found in the literature (Fig. 1). In an experiment with a single dose of 2 g, analogous results were obtained.

Elimination of MTU after ingestion of multiple doses

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

After 2, 3 or 4 week of 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 two phases in the disappearance of MTU from plasma or urine (Fig. 2).

In the first 4–5 d after stopping MTU treatment, the MTU levels in plasma and urine declined rapidly, then the elimination rate decreased by a factor of 20 so that, after a withdrawal period of more than 60 d, still distinct levels of MTU (≥ 10 ppb) were found in urine or plasma. During that period the residue levels in urine were approximately five times higher than those in the corresponding plasma samples. There were no significant differences in the elimination constants after feeding MTU to cows for either 2, 3 or 4 weeks.

After feeding cows with MTU for longer periods (14–28 d), the initial elimination of MTU from plasma, urine or tissues was 2.5–3.3 times slower than that 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



Fig. 2 Time course of MTU levels in plasma and urine of a cow after oral ingestion of a daily dose of 5 g of MTU for 21 d.

Table 1Half-life of MTU in plasma, urine, muscle and thyroid tissue afteroral administration of the drug to cattle as calculated with the MW/Pharm(Kinfit) program

Dose ^a	Fluid or tissue	Half-life of MTU/d ^b		
		First phase	Second phase	DT ^c /d
Single dose				
1×2 or 4 g	urine, milk (2)	5.7		
1 × 5 g	plasma (8)	4.8		16
$1 \times 5 \text{ g}$	Thyroid (8)	2.8		29
Multiple dose				
14, 21 or 28	plasma, urine (13)	10	12	65
28×5 g	thyroid (13)	9.9		77
	diaphragm (10)	9.9		66

 a ×, number of animals. ^b Estimated from analysis of samples obtained at slaughter after withdrawal times of at least 3 d. ^c Withdrawal period (days) during which the concentration of MTU residues in the biological matrix exceeds 0.01 ppm.

MTU in thyroid and diaphragm probably indicate that cow tissues reached steady-state levels after feeding MTU for at least 14 d.

MTU concentrations in the thyroid and meat

The MTU concentrations in the thyroid were studied over a withdrawal period of 5–80 d. During this period, the apparent half-life of MTU in this organ was at least three times shorter than that observed in plasma or urine over the same period (Table 1). If all data are plotted (Fig. 3), two regression lines with different slopes are obtained. The regression coefficient obtained for meat (0.677) is smaller than that for thyroid (0.936). However, it was observed that the MTU concentrations found in meat during the first 20 d of the withdrawal period were lower than could be calculated from the regression line. In Fig. 4 it is shown that the ratio of the MTU concentration in the thyroid to that in meat decreases considerably (from about 150 at 3 d to 1–3 after 20 d withdrawal) with the withdrawal period; the thyroid shows a specific and efficient accumulation of MTU over that of other muscular tissues.

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 (if the outliers are removed). The MTU residue levels in the thyroid were three times higher than that of M. diaphragma over the withdrawal period studied. From this study, it is evident that after MTU treatment of cows for 4 weeks followed by a withdrawal period of 1 month, still appreciable residue levels (about 0.1 ppm MTU) may be found in muscular tissue. MTU treatment therefore results in higher residue concentrations in muscular tissue during longer withdrawal periods than was expected from earlier experiments.^{16–18}

Thyroxine levels in animals treated with MTU

The serum levels of thyroxine (T_4) were studied in six cows during a 3 or 4 week MTU treatment. Normal T_4 values ranged



Fig. 3 Concentration of MTU levels in the thyroid and the diaphragm of cows.



Fig. 4 Ratio of MTU level in the thyroid to that in the diaphragm.

between 9 and 20 μ g of T₄ per 100 ml of serum. After 10–14 d of MTU treatment the T₄ values decreased. The minimum values ranged between 3.2 and 7.8 μ g of T₄ per 100 ml of serum from 16 to 22 d after starting the MTU treatment. Similar observations were made by Pohlschmidt and Forschner¹⁸ after feeding MTU to seven bulls over a period of 26 d. However, their minimum T₄ values were lower (1–2 μ g of T₄ per 100 ml of serum). The time course of the T₄ values during the experiments were similar if the T₄ content was expressed as a percentage of its initial value: minimum T₄ values were 37 ± 3% of the initial values (Fig. 5).

Three days after withdrawal of the drug, the T₄ content of serum increased and reached a maximum 5-6 d after stopping the MTU treatment. Then the T₄ content declined to 70% of its original value, after which the T₄ values started to oscillate. Prolonged administration of goitrogenic substances to cattle results in symptoms of hypothyroidism: histological hypertrophy of the thyroid gland epithelium or macroscopic enlargement of the thyroid gland (mass). Enlargement of the thyroid (by a factor of 2–3) in comparison with the normal thyroids of other animals from the same farm was also observed in these experiments. These parameters 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 for 1 month will not result in a sufficiently long period of hypothyroidism to induce 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.19

4 Conclusion

Treatment of cows with MTU results in the rapid appearance of the drug in plasma, urine and milk As could be expected, thyreostats such as MTU selectively accumulate in the thyroid. MTU levels in plasma or urine show a biphasic elimination: after an initial rapid decline, observed in the first 4 d after treatment, the MTU excretion slows by a factor of 15–30 depending on the treatment. Feeding MTU to cows over prolonged periods results in slower MTU elimination so that, after a withdrawal period of 1 month, appreciable residue levels of the drug were found in the thyroid, muscular tissues, urine and plasma.

The time course of serum thyroxine levels during MTU treatment of cows suggests that histological or macroscopic examination of the thyroid cannot be used as a reliable screening method 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. For regulatory control on the living animal, urine sampling is the obvious choice. Only limited experience in the determination of the drugs in faeces is



Fig. 5 Time course of T_4 serum levels during and after treatment of three cows with 5 g of MTU d⁻¹ for 28 d. The T4 values are expressed as percentages of the initial control values.

available.²⁰ During inspection in the slaughterhouse, the thyroid should be selected as the target tissue for optimum detection of the illegal use of thyreostatics. In comparison with earlier data and methods,^{16–18,21} the HPTLC detection method employed for the animal experiments allowed the detection of significant MTU levels in the thyroid, muscular tissue, plasma or urine over a 10-fold extended withdrawal period. The HPTLC method is sufficient for regulatory control since residues of the drugs could be detected much longer than the mass-gain effect of the drug. However, in many laboratories TLC experience is lost and modern instrumental methods such as GC or LC-MS are preferred. The recently developed methods for thyreostats of the MTU series based on modern instrumentation²²⁻²⁶ are well suited for the control of the abuse of these drugs. Moreover, the combination of the excretion data with the lower detection limits suggests that the 'detection period' may become so long that animals may stay 'positive' months after treatment. When animals have been in the possession of several owners, the indication of which owner administered the drug is more within the scope of a detective than an analyst.

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