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The transfer of arsenic to sheep tissues

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SUMMARY

There is the potential for arsenic to enter the human food chain via ingestion by grazing animals. Data on the transfer of arsenic to ruminants have been too sparse to allow the development of dynamic models to predict changes in the arsenic contents of different tissues following ingestion. A study is described during which a group of 6-month-old lambs were given a single oral administration of $^{73}AsCl_3$. Subsequently, concentrations of ^{73}As in the tissues of groups of lambs slaughtered at intervals over a period of 181 days were determined. A true absorption coefficient of $0{\cdot}46 \pm 0{\cdot}055$ (mean $\pm$ S.E.) was determined which is considerably lower than expected from previous studies of non-ruminant animals which demonstrate complete absorption for inorganic arsenic. The resultant data were used to develop a compartment model to describe arsenic behaviour in sheep tissues. The derived model accounted for 80 % ($n = 100$) of the observed variation in the data. The model predicts that arsenic concentrations in tissues rapidly (< 40 days) reach equilibrium with the dietary intake level. Equilibrium transfer coefficient values (the ratio of the arsenic concentration in a tissue to the daily dietary intake of arsenic) for the important food-chain tissues were calculated as: $(2{\cdot}5 \pm 0{\cdot}67) \times 10^{-3}$ days/kg for muscle, $(9{\cdot}1 \pm 1{\cdot}96) \times 10^{-3}$ days/kg for liver and $(1{\cdot}1 \pm 0{\cdot}14) \times 10^{-2}$ days/kg for kidney.

INTRODUCTION

Arsenic is a metalloid element, ubiquitous within the environment. It is toxic and can also have carcinogenic, teratogenic and genetic effects (Fowler *et al.* 1979; Léonard & Lauwerys 1980). It may be released into the environment by a number of industrial processes such as the refining of non-ferrous ores and combustion of fossil fuels (Pacnya 1987) resulting in localized enhanced levels (Adamson 1980; MAFF 1982). Arsenic has also had a number of uses within the agricultural industry including incorporation within animal feeds as a growth promoter in pig and poultry production (Calvert & Smith 1981; Monies 1999), herbicides, pesticides and wood preservers (Adriano 1986; Hullinger *et al.* 1998). The agricultural uses of arsenic have declined over recent years in response to legislative changes, although cases of exposure of farm livestock to arsenic resulting from stored and discarded chemicals still occur (Stair *et al.* 1995; Monies 1999). The spreading of fly ash and poultry wastes onto agricultural land also represents a potential source of contamination (Jackson & Miller 1998). Particularly high, localized arsenic contamination occurs in some areas of historic mining and metal ore smelting (Abrahams & Thornton 1994; Davis *et al.* 1996; Koch *et al.* 1999). Whilst rare, the death of livestock as a result of arsenic poisoning has been recorded in such areas (Thomas 1980). Cases of pollutant contamination of commercially prepared animal feeding stuffs have also occurred. For instance, within Europe during 1989–1990 imported animal feed contaminated with a number of heavy metals was allowed to enter the food chain. Although lead was the prime contaminant of concern, levels of other heavy metals, including arsenic, were in excess of national guideline values (Baars *et al.* 1992; Crews *et al.* 1992).

It can be seen that there are a number of circumstances by which farm animals may be exposed to arsenic thus presenting a potential route of transfer to humans via the food chain. Consequently, there is a requirement to be able to predict accurately the transfer of arsenic to animal-derived food products and also the rates of decline of arsenic in the tissues of

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exposed animals. Within the literature, there are monitoring data on the levels of arsenic in animal tissues (e.g. Vos *et al.* 1987, 1988) and some experimental studies from which values for the transfer of arsenic from feed to tissues/milk can be derived (Calvert & Smith 1981; van der Veen & Vreman 1986; Vreman *et al.* 1986; Stevens 1991; Stevens 1992). However, there are insufficient data to develop time-dependent food chain models.

In the current paper experimental and modelling studies to derive a model of the transfer of arsenic to the tissues of sheep are described.

EXPERIMENTAL STUDIES

Materials and methods

Thirty Scottish Blackface lambs aged 6–7 months, were individually housed and fed a diet of dried grass pellets (900 g dry matter (DM) per day) and alkali-treated straw pellets (196 g DM per day) for a period of approximately 4 weeks prior to the start of the study.

The lambs were placed in metabolism cages and allocated to 10 groups of three on the basis of anticipated slaughter dates of 0·5, 1·5, 3, 6, 10, 16, 24, 45, 90 and 181 days after radioisotope administration. On a single occasion (Day 0), a carrier free solution containing $^{73}AsCl_3$ (John Caunt Scientific, Eynsham, Oxon., UK) was administered to each lamb directly into the rumen via a tube passed down the oesophagus. Animals intended for slaughter before Day 90 were administered 0·60 MBq ^{73}As; because of its short radioactive half-life (80 days) the administered activity of ^{73}As was increased by a factor of two and four for animals anticipated to be slaughtered on Day 90 and Day 180 respectively. For the purposes of a separate study on the transfer of cadmium and mercury to sheep tissues (Beresford *et al.* 1999) the animals were also administered solutions containing ^{109}Cd and ^{203}Hg concurrent to the ^{73}As.

Total faeces and urine outputs were collected from 12 of the animals (allocated to later slaughter dates) for 21 days after radioisotope administration. Sub-samples of excreta were weighed into 700 ml or 2·3 l plastic containers and stored at −20 °C to await analysis. Blood samples (*c.* 24 ml) were collected, by venepuncture into heparinized tubes, from the jugular vein of three of these animals at 1 h, 2 h, 4 h, 8 h, 12 h, 30 h, 2 days, 3 days, 4 days, 7 days, 14 days and 28 days after ^{73}As administration.

Immediately prior to slaughter by captive bolt each animal was weighed. After collection of a sample of blood (> 2l, mixed with heparin to prevent clotting), the liver and kidneys were removed together with samples of muscle (hind leg) and bone (femur). Lungs, mesenteric fat and skin were sampled up to Day 10; reticulo-rumen contents and combined intestinal and caecal contents (hind gut) were sampled up to Day 24. Sheep remaining after Day 24 were transferred from metabolism cages to individual pens.

To determine the proportion of liveweight attributable to muscle, bone and fat, total dissections on a half of the carcass (divided down the spine) were performed from one sheep slaughtered on each of 1·5, 6, 16, 90 and 181 days after radioisotope administration. On three occasions during the study, the fleece was removed by shaving, and the skin removed and weighed.

Blood samples were centrifuged (2200 ***g*** for 20 min) to separate plasma and red blood cells (RBC); the RBC were washed by resuspension in 0·15 M NaCl solution followed by centrifugation (2200 ***g*** for 20 min). In addition, for blood samples taken at

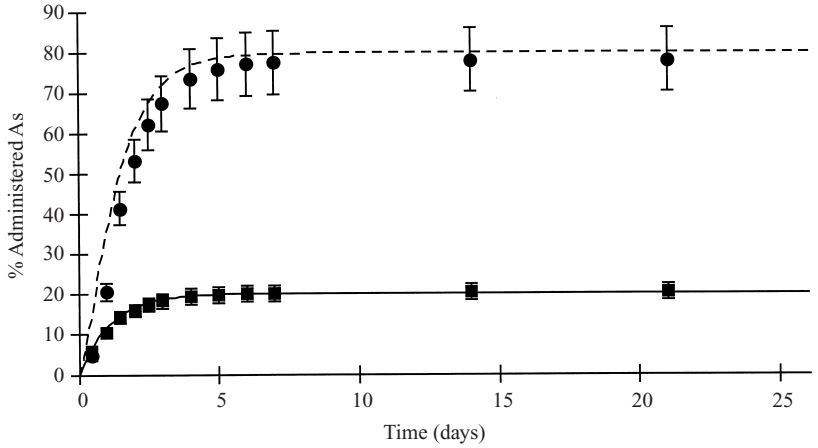


Fig. 1. Observed (mean ± S.E.) and modelled cumulative excretion of ^{73}As to faeces (●– – –) and urine (■——) as a function of time since administration.

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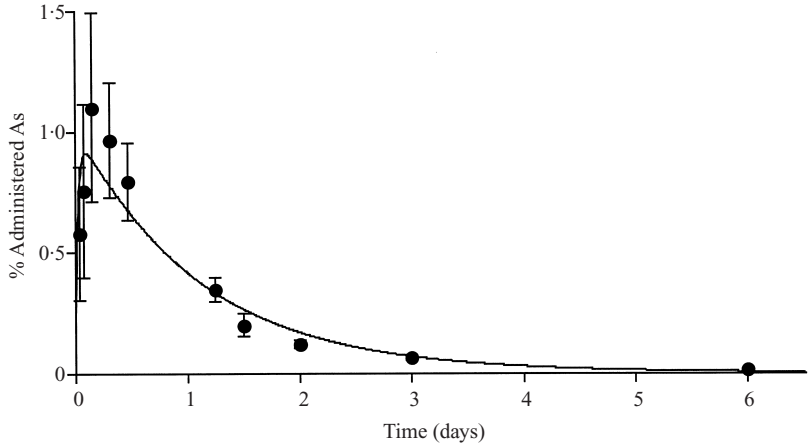


Fig. 2. Observed (mean ± S.E.) and modelled content of ^{73}As in plasma as a function of time since administration.

slaughter, the density of whole blood was estimated and packed cell volume (PCV) determined using micro-haematocrit tubes. Soft tissue samples were sliced into approximately 1 cm^3 pieces and well mixed before being weighed into plastic containers (130 ml to 2·3 l). All samples were stored at −20 °C.

Approximately 400 g of fresh bone, free from soft-tissue, was dissolved in 400 ml of concentrated HNO_3 and diluted to 700 ml with deionized water.

The amounts of feed offered were increased to 1213 g DM per day after Day 90 maintaining the same grass pellet: straw pellet ratio. Samples of feed were retained throughout the study.

Sample analyses

The activity concentrations of ^{73}As in tissue and excreta samples were determined using hyper-pure germanium detectors. Resultant spectra were analysed using Canberra *Apogee* software. The detectors were calibrated using mixed gamma standards of appropriate matrix and volume. Analyses times ranged from 1000 to 240000 seconds depending upon the individual sample activity; generally, a counting error of less than 5% of the result was achieved. The date of isotope administration was used as the reference date to correct for radioactive decay. Plasma and RBC samples were made up to a standard volume with deionized water prior to analysis.

After freeze-drying, subsamples of the dietary components were digested in concentrated nitric acid (BDH Aristar) under reflux. The arsenic concentrations in diluted aliquots of the digests were determined by inductively coupled plasma mass spectrometry. Limits of detection were taken to be three times the standard deviation of determinations conducted on 18 blank samples prepared at the same time as the sample digests (Miller & Miller 1988). To ensure adequate quality control a certified ryegrass reference sample was analysed at the same time as the samples (Community Bureau of Reference CRM281).

Experimental results

The cumulative outputs of ^{73}As in faeces and urine obtained from the 12 animals sampled over the first 3 weeks after radioisotope administration are presented in Fig. 1. The total outputs after 3 weeks amounted to 78 ± 3·9% (mean ± S.E.) of the administered activity in faeces and 21 ± 1·5% in urine. If it is assumed that all of the ^{73}As being excreted after Day 8 was of endogenous origin, the endogenous faecal excretion (EF) of ^{73}As over the 21-day period of excreta collection can be estimated (see Mayes *et al.* 1996):

EF ^{73}As (Bq/day)
= (Total urinary ^{73}As output 0−21 days (Bq)
× Faecal ^{73}As output 8−21 days (Bq))/
(Urinary ^{73}As output 8−21 days (Bq))

The percentage of administered ^{73}As estimated to have been endogenously excreted via faeces was 24·3 ± 5·18% (mean ± S.E.; $n = 12$). Using this value for EF, the true absorption coefficient (A_t) (Mayes *et al.* 1996) of ^{73}As can be calculated:

A_t = (^{73}As Intake (Bq) − Faecal ^{73}As output (Bq)
+ EF ^{73}As (Bq))/(^{73}As Intake (Bq))

The value of A_t for ^{73}As estimated by this method was 0·46 ± 0·055 (mean ± S.E.; $n = 12$). With the exception of one animal, for which an A_t value of 0·93 was determined, A_t estimates were in the range 0·19–0·61.

The activity concentration of ^{73}As in plasma

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Table 1. *The activity concentration* (*mean* ± *S.E.*) *of* ^{73}As, *normalized to the activity administered, in sampled tissues throughout the study* (*per kg*)

	Day 0·5	Day 1·5	Day 3	Day 6	Day 10	Day 16	Day 24	Day 45	Day 90	Day 181	Day 365
Muscle	9·25E−04	2·76E−04	1·77E−04	6·42E−05	5·35E−05	3·04E−05	2·60E−05	1·72E−05	8·22E−06	2·91E−06	< 7·52E−6
	1·635E−04	2·26E−05	3·40E−05	8·40E−06	1·556E−05	6·34E−06	8·75E−06	2·00E−06	1·288E−06	3·76E−07	
Liver	5·37E−03	1·73E−03	1·05E−03	3·85E−04	2·09E−04	1·51E−04	1·05E−04	1·46E−05	2·09E−05	< 1·58E−5	< 2·84E−5
	1·442E−03	2·67E−04	2·08E−04	9·29E−05	4·81E−05	5·38E−06	3·43E−05	3·10E−06	6·44E−06		
Kidney	7·33E−03	2·63E−03	9·27E−04	2·39E−04	7·86E−05	5·07E−05	3·42E−05	< 4·00E−5	< 2·58E−5	< 6·02E−5	< 4·18E−5
	6·13E−04	1·91E−04	9·52E−05	3·61E−05	2·170E−05	1·186E−05	1·681E−05				
Plasma	1·55E−03	3·59E−04	1·21E−04	2·20E−05	< 2·16E−5	< 2·68E−5	< 4·08E−5	1·41E−05	< 1·95E−5	< 2·71E−5	< 2·78E−5
	2·89E−04	6·54E−05	1·79E−05	2·96E−06				< 2·22E−06			
RBC	7·21E−04	2·11E−04	8·24E−05	3·63E−05	1·71E−05	9·468E−06	< 2·16E−5	< 4·92E−5	< 1·74E−5	< 1·15E−5	< 2·84E−5
	1·091E−04	3·56E−05	2·053E−05	8·81E−06	4·16E−06	2·30E−06					
Bone	4·96E−04	2·36E−04	2·01E−04	9·83E−05	< 2·62E−4	< 7·90E−4	< 2·81E−4	< 2·59E−4	< 1·26E−4	< 1·04E−4	< 5·70E−5
	1·103E−04	3·80E−05	7·82E−05	1·529E−05							
Lung	2·28E−03	6·22E−04	2·98E−04	8·22E−05	3·84E−05						
	1·72E−04	6·16E−05	3·45E−05	7·95E−06	4·37E−06						
Mesenteric fat	4·29E−04	2·52E−04	1·80E−04	5·17E−05	3·74E−05						
	8·60E−05	2·80E−05	1·79E−05	7·20E−06	2·429E−05						
Skin	1·33E−03	4·96E−04	3·15E−04	1·38E−04	4·09E−04						
	1·51E−04	8·00E−05	8·68E−05	4·38E−05	7·03E−05						

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Table 2. *The percentage (mean ± S.E.) of administered ^{73}As in sampled tissues throughout the study*

	Day 0·5	Day 1·5	Day 3	Day 6	Day 10	Day 16	Day 24	Day 45	Day 90	Day 181	Day 365
Muscle	6·43E−01	1·96E−01	1·23E−01	4·51E−02	3·84E−02	2·27E−02	2·02E−02	1·39E−02	7·57E−03	3·40E−03	< 9·04E−3
	1·181E−01	1·94E−02	2·57E−02	5·05E−03	1·203E−02	5·24E−03	8·02E−03	1·683E−03	1·193E−03	4·97E−04	
Liver	2·49E−01	9·33E−02	5·60E−02	1·99E−02	1·07E−02	7·11E−03	5·30E−03	7·19E−04	1·14E−03	< 1·02E−3	< 1·68E−3
	6·957E−02	1·587E−02	7·98E−03	4·84E−03	2·60E−03	4·26E−04	1·888E−03	1·594E−04	6·40E−04		
Kidney	7·52E−02	2·75E−02	8·71E−03	2·76E−03	8·99E−04	5·77E−04	3·90E−04	< 5·44E−4	< 3·64E−4	< 7·14E−4	< 4·80E−5
	7·70E−03	1·21E−03	9·06E−04	4·41E−04	2·491E−04	1·467E−04	1·901E−04				
ECF	8·25E−01	1·99E−01	6·44E−02	1·18E−02	< 5·10E−3	< 7·01E−3	< 1·10E−2	8·66E−03	< 6·86E−3	< 1·21E−2	< 2·8E−2
	1·620E−01	4·77E−02	1·021E−02	1·413E−03				1·33E−03			
RBC	6·11E−02	1·89E−02	7·26E−03	3·14E−03	1·49E−03	8·66E−04	< 2·08E−3	< 5·36E−3	< 1·52E−3	< 1·58E−3	< 3·48E−3
	9·144E−03	3·61E−03	1·530E−03	5·70E−04	3·28E−04	1·464E−04					
Bone	1·07E−01	5·17E−02	4·37E−02	2·46E−02	< 6·00E−2	< 1·92E−1	< 7·19E−2	< 6·46E−2	< 3·40E−2	< 3·92E−2	< 2·08E−2
	6·19E−02	2·985E−02	2·525E−02	1·421E−02							
Lung	6·64E−02	2·51E−02	9·93E−03	2·84E−03	1·33E−03						
	7·17E−03	3·25E−03	1·723E−03	3·03E−04	1·92E−04						
Fat	1·06E−01	6·37E−02	4·43E−02	1·30E−02	9·76E−03						
	2·11E−02	8·27E−03	4·00E−03	1·95E−03	6·593E−03						
Skin	2·95E−01	1·10E−01	7·01E−02	3·09E−02	6·50E−02						
	3·58E−02	7·53E−02	8·907E−02	1·153E−02	2·833E−02						
Reticulo-	4·19E+01	2·71E+01	3·17E+00	5·89E−01	7·27E−03	< 1·30E−2	< 4·24E−3				
rumen	9·62E+00	1·46E+01	5·01E−01	1·63E−01	1·38E−03						
Intestine	2·15E+01	9·70E+00	1·99E+00	2·64E−01	8·27E−03	2·13E−03	1·37E−03				
	4·66E+00	1·280E+00	1·08E−01	5·35E−02	3·010E−03	1·022E−03	2·22E−04				

derived from blood samples collected from the jugular vein of the three animals sampled, reached maximum values 4 h after administration (Fig. 2). Activity concentrations were below the minimum detectable activity in samples collected later than 48 h after administration (the small size of these samples (< 10 g) resulted in a higher than usual detection limit).

To remove the effect of increases in the amount of administered ^{73}As with anticipated slaughter date, results for tissue samples have been expressed as the activity concentration normalized to the activity administered (Table 1). A value of half the detection limit was assumed for the samples below the detection limit in order to calculate the mean and S.E. values when results for a given day included measurements both above and below the detection limit. Peak activity concentrations of ^{73}As were measured after 12 h in all of the sampled tissue types. Thereafter, concentrations declined, reaching minimum detection limits in most tissues by Day 45 (Table 1). Arsenic-73 activity concentrations were initially highest in the kidney. Concentrations in this organ fell rapidly and between 7 and 14 days after administration were lower than those in liver, which was initially the second most contaminated organ.

To calculate the percentage of administered activity within a given tissue (Table 2), the organ weights measured at slaughter were used for kidney, liver and lung. Total weights of plasma and red blood cells were calculated, on the basis of published live-weight to blood volume relationships (Hansard *et al.* 1953) and whole blood density and PCV measurements. The total amounts of administered isotopes in extracellular fluid (ECF) were estimated, assuming that the activity concentration measured in plasma was representative of this pool and that ECF comprised 18% of the animals' liveweight (Ladd Prosser 1991). The percentages of liveweight, as calculated from the half-carcass dissections, which were attributable to muscle (23·6%), bone (7·4%) and fat (8·4%) were used to estimate the total mass of these tissues throughout the study. The activity concentrations of ^{73}As in mesenteric fat were assumed to be representative of all body fat pools. From measurements made on three animals, it was estimated that skin represented 7·5% of the animals' live-weight. The mean liveweights of the animals at slaughter increased as the study progressed from $29 \pm 0{\cdot}5$ kg on Day 0·5 to $50 \pm 0{\cdot}9$ kg on Day 181. On early sampling occasions, muscle and ECF were estimated to contain the largest percentages of absorbed ^{73}As. However on Day 0·5 *c.* 64% of the administered ^{73}As was still present in the reticulo-rumen and intestine; by Day 1·5 this proportion had declined to *c.* 35%. By Day 10 the highest percentage was estimated to be in skin; skin samples were not analysed after Day 10.

The concentration of stable arsenic in grass pellets was in the range $< 0{\cdot}2$ to 0·3 mg/kg DM; concentrations in pelleted straw were below detection limits ($< 0{\cdot}2$ mg/kg DM).

MODELLING STUDIES

Modelling methods

The models were solved numerically using the 4th order Runge–Kutta method (Press *et al.* 1986) and the unknown model parameters were estimated by fitting to the experimental data. A frequent difficulty with parameterizing models of this type is that standard non-linear fitting procedures (e.g. Press *et al.* 1986) can become trapped in local minima on the sum of squares surface (i.e. the lowest sum of squares value is not found). The local minima reached can be dependent upon initial parameter estimates (i.e. a different local minima and consequently parameter value may be derived for different initial values). To avoid this the Marquardt method was adapted in a fashion analogous to simulated annealing (Ingber 1993). This stochastic approach allows the fitting procedure to avoid local minima thereby reducing the dependence of the estimated parameters on their initial estimates.

To account for the different magnitude of the data and differences in experimental uncertainty for the various compartments, the fitting procedure minimized the weighted sum of square residuals. For each data point, the weighting factor was the reciprocal of its standard error estimated from sample replicates.

Model development

The model structure (Fig. 3) was an adaptation of that previously employed to describe the behaviour of a number of heavy metals and radionuclides of metallic elements in sheep tissues (Galer *et al.* 1993; Beresford *et al.* 1998; Beresford *et al.* 1999). In these earlier models, each tissue was represented as a single compartment. However, in this case a similar structure was not able to describe the observed dynamic behaviour of arsenic in muscle, liver and kidney. The experimental results showed a rapid initial rise and fall in activity combined with a long slow decline. To account for this, the compartments for these tissues were subdivided (justification for this is given in the Discussion) as shown in Fig. 3. These changes were implemented sequentially, at each stage the significance of any improvement in fit was tested (F-test) as summarized in Table 3. The inclusion of extra compartments gives a small, but significant, improvement in the model residual for muscle, liver and kidney. The subsequent partitioning of other tissue compartments was considered but none was found

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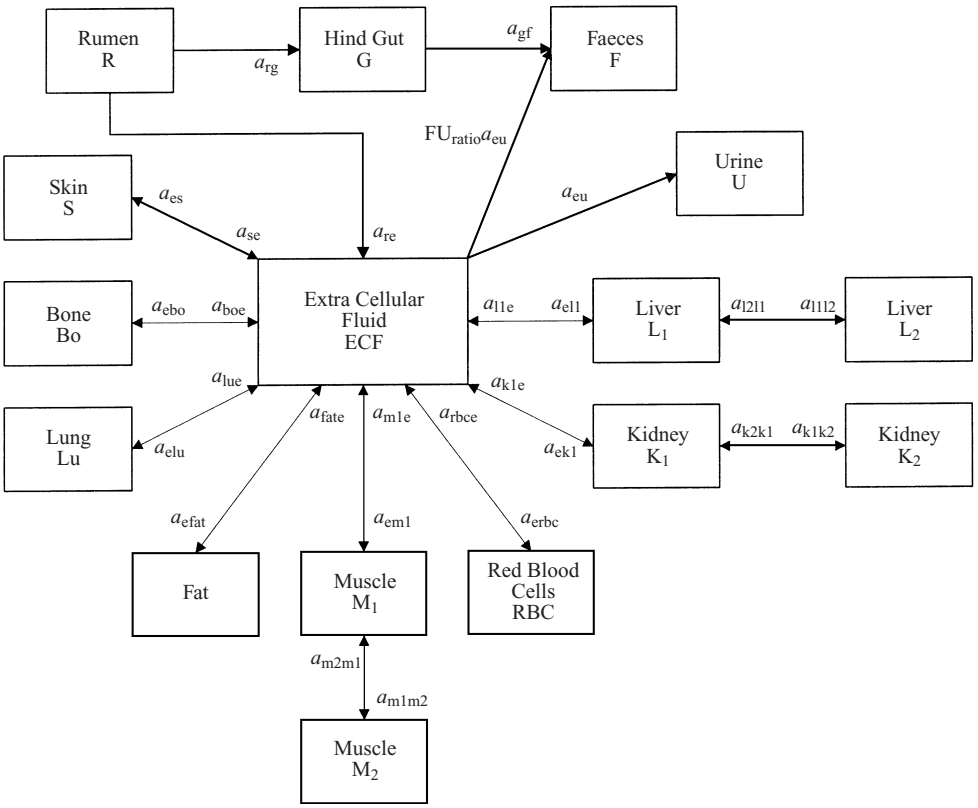


Fig. 3. Schematic representation of the developed multi-compartment model. Symbols used for each compartment are shown together with the rate coefficients controlling the transfers between compartments.

Table 3. *Accumulated analysis of variance for the successive model fits as extra compartments for the muscle, liver and kidney compartments are added*

	D.F.	SS (r^2)	MS	*F*	*P*
Initial model*	19	1675·8 (0·71)	88·2	14·1	< 0·001
+ subdivided muscle	2	109·0 (0·76)	54·5	8·70	< 0·001
+ subdivided muscle and liver	2	50·9 (0·78)	25·5	4·08	0·021
+ subdivided muscle, liver and kidney	2	49·9 (0·80)	24·0	3·84	0·022
Residual	74	462·3	6·25		
Total	99	2347·9			

* Single tissue compartment model.

significantly to improve the fit. A further change from the earlier models is that absorption is assumed to occur from the rumen rather than the hind gut. During model parameterization it was found that the coefficient for transfer from the hind gut was not significantly different from zero. Therefore transfer from the hind gut was omitted from the final model. This is probably as a consequence of the very rapid rise in ^{73}As concentrations in plasma (Fig. 2). Arguably endogenous excretion should be included as a transfer between ECF and both the rumen and/or hind gut. However, the model was parameterized using the experimentally observed ratio of endogenous faecal to urinary excretion (1·157). This was felt to be a more robust approach given that it utilizes directly observed values rather than estimating further coefficients through model parameterization.

The model comprises a set of simultaneous first

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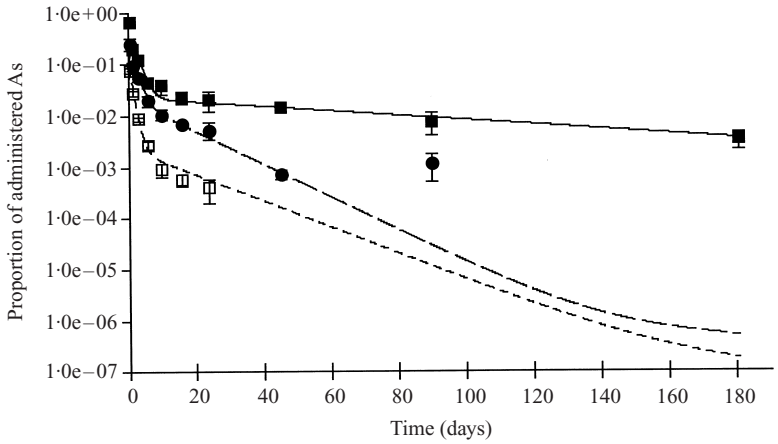


Fig. 4. Observed (mean ± S.E.) and modelled ^{73}As content of muscle (■——), liver (●– – –) and kidney (□- - - -) as a function of time since administration.

Table 4. *Contribution of individual compartments to the total weighted residual sum of squares (WRSS)*

Compartment	WRSS	% Total WRSS
Faeces	107	23
Urine	3·4	0·73
Muscle	21	4·6
Liver	10	2·2
Kidney	4·0	0·87
Lung	89	19
RBC	60	13
ECF	34	7·4
Fat	14	3·1
Rumen	35	7·5
Hind gut	67	14·5
Skin	17	3·6
Bone	1·7	0·36

order differential equations describing the rate of change of arsenic in various compartments representing the tissues of the animal:

$$\frac{dR}{dt} = -a_{rg}R - a_{re}R$$

$$\frac{dG}{dt} = a_{rg}R - a_{gf}G$$

$$\frac{dECF}{dt} = a_{re}R + a_{l1e}L_1 + a_{m1e}M_1 + a_{k1e}K_1 + a_{lue}Lu + a_{boe}Bo + a_{se}S + a_{rbce}RBC + a_{fate}FAT - (a_{el1} + a_{ek1} + a_{em1} + a_{elu} + a_{ebo} + a_{erbc} + a_{es} + a_{efat} + (1 + FU_{ratio})a_{eu})ECF$$

$$\frac{dM_1}{dt} = a_{em1}ECF - (a_{m1e} + a_{m1m2})M_1 + a_{m2m1}M_2$$

$$\frac{dM_2}{dt} = a_{m1m2}M_1 - a_{m2m1}M_2$$

$$\frac{dL_1}{dt} = a_{el1}ECF - (a_{l1e} + a_{l1l2})L_1 + a_{l2l1}L_2$$

$$\frac{dL_2}{dt} = a_{l1l2}L_1 - a_{l2l1}L_2$$

$$\frac{dK_1}{dt} = a_{ek1}ECF - (a_{k1e} + a_{k1k2})K_1 + a_{k2k1}K_2$$

$$\frac{dK_2}{dt} = a_{k1k2}K_1 - a_{k2k1}K_2$$

$$\frac{dLu}{dt} = a_{elu}ECF - a_{lue}Lu$$

$$\frac{dBo}{dt} = a_{ebo}ECF - a_{boe}Bo$$

$$\frac{dRBC}{dt} = a_{erbc}ECF - a_{rbce}RBC$$

$$\frac{dS}{dt} = a_{es}ECF - a_{se}S$$

$$\frac{dFat}{dt} = a_{efat}ECF - a_{fate}Fat$$

$$\frac{dF}{dt} = a_{gf}G + FU_{ratio}a_{eu}ECF$$

$$\frac{dU}{dt} = a_{eu}ECF$$

where:

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the a values are the model's rate coefficients (per day), the subscripts representing the transfer process they apply to (e.g. a_{em}, ECF → muscle); R, G, ECF, M_1, M_2, L_1, L_2, K_1, K_2, Lu, Bo, RBC, S, Fat, F and U are the contents of arsenic (Bq in the case of the experimental data) in the reticulo-rumen, hind gut, extracellular fluid, muscle, liver, kidney, lung, bone, red blood cells, skin, fat, faeces and urine compartments respectively; subscripts 1 and 2 for muscle, liver and kidney refer to fast and slow pools respectively; FU_{ratio} is the experimentally observed ratio of endogenous excretion of arsenic via faeces to that via urine (1·157).

Model results

Model predictions for excreta, plasma, muscle, liver and kidney are compared with experimental results in Figs 1, 2 and 4. The only notable outlying point is the final liver value (Day 90) which is underpredicted by the model. It is difficult to comment on whether it is a data anomaly given that ^{73}As activity concentrations in subsequent liver samples were below the limit of detection.

The model has a weighted sum of squared residuals of 462·3 ($n = 100$) and accounts for 80 % of the total variation in the observed data, the partitioning of the weighted sum of squared residuals between compartments is shown in Table 4. The estimated rate coefficients are given in Table 5. In some cases the estimated values have large S.E.S. This is due to high correlation between the estimation of some parameter pairs, for example the extreme case is the correlation coefficient of 0·99 between a_{k1e} and a_{ek1}, the rate coefficients describing transfer between ECF and the kidney. Within the fitting process a change in a_{k1e} can be offset by a change in a_{ek1}. Whilst this is not a desirable feature of the model it need not affect the uncertainty in predictions unduly as the effect is offset by a correspondingly high covariance between these parameters. A high correlation is only observed for parameter pairs describing transfer in and out of a given tissue (Table 5). The remaining parameters are independent.

DISCUSSION

Many previous authors (see reviews by Fowler *et al.* 1979; Morgan 1991; Gompertz *et al.* 1997) have stated that the predominant excretion route of absorbed arsenic in animals, including ruminants (Peoples 1964), is urinary. A few authors have disagreed, suggesting that endogenous faecal excretion will be important for inorganic As(III) (Schroeder & Balassa 1966; NRC 1980). The findings of the current study, in which endogenous faecal excretion and urinary excretion of arsenic were comparable, support the latter suggestion. Biswas *et al.* (1998) report that faeces was the predominant excretion route of arsenic in goats; however, animals were administered sodium arsenite at a daily intake rate which eventually achieved 100 % mortality.

There do not appear to be any reported values for arsenic absorption in ruminants. However, on the basis of a review of data for non-ruminant animals, Morgan (1991) suggested that a fractional gastrointestinal absorption value of 0·95 be assumed for inorganic trivalent and pentavalent arsenic. The mean true absorption coefficient of As(III) determined

Table 5. *Estimated model rate coefficients and standard errors*; *correlation coefficients for parameter pairs controlling transfer in and out of a given tissue as also presented*

Rate coefficient	Estimate (per day)	S.E. (per day)	Rate coefficient	Estimate (per day)	S.E. (per day)	Correlation coefficient
a_{boe}	$3{\cdot}38 \times 10^{-1}$	$5{\cdot}20 \times 10^{-1}$	a_{ebo}	$1{\cdot}08 \times 10^{-1}$	$1{\cdot}38 \times 10^{-1}$	0·89
a_{fate}	$5{\cdot}43 \times 10^{-1}$	$1{\cdot}56 \times 10^{-1}$	a_{efat}	$1{\cdot}33 \times 10^{-1}$	$5{\cdot}14 \times 10^{-2}$	0·77
a_{k1e}	33·8	83·8	a_{ek1}	3·47	8·39	0·99
a_{k1k2}	$2{\cdot}61 \times 10^{-2}$	$2{\cdot}28 \times 10^{-2}$	a_{k2k1}	$1{\cdot}08 \times 10^{-1}$	$7{\cdot}34 \times 10^{-2}$	0·91
a_{l1e}	1·11	1·07	a_{el1}	$3{\cdot}04 \times 10^{-1}$	$3{\cdot}00 \times 10^{-1}$	0·97
a_{l1l2}	$7{\cdot}07 \times 10^{-2}$	$4{\cdot}16 \times 10^{-2}$	a_{l2l1}	$8{\cdot}34 \times 10^{-2}$	$2{\cdot}23 \times 10^{-2}$	1·00
a_{m1e}	$6{\cdot}67 \times 10^{-1}$	$2{\cdot}56 \times 10^{-1}$	a_{em1}	$4{\cdot}47 \times 10^{-1}$	$1{\cdot}84 \times 10^{-1}$	0·90
a_{m1m2}	$3{\cdot}50 \times 10^{-2}$	$1{\cdot}88 \times 10^{-2}$	a_{m2m1}	$1{\cdot}27 \times 10^{-2}$	$7{\cdot}64 \times 10^{-3}$	−0·05
a_{rbce}	$1{\cdot}88 \times 10^{-1}$	$5{\cdot}81 \times 10^{-2}$	a_{erbc}	$9{\cdot}16 \times 10^{-3}$	4·50	0·90
a_{se}	$8{\cdot}68 \times 10^{-1}$	$4{\cdot}15 \times 10^{-1}$	a_{es}	$8{\cdot}24 \times 10^{-1}$	$3{\cdot}60 \times 10^{-1}$	0·57
a_{lue}	$6{\cdot}58 \times 10^{-1}$	$1{\cdot}31 \times 10^{-1}$	a_{elu}	$5{\cdot}01 \times 10^{-2}$	$2{\cdot}00 \times 10^{-2}$	0·92
a_{rg}	$5{\cdot}34 \times 10^{-1}$	$3{\cdot}66 \times 10^{-2}$				
a_{re}	$4{\cdot}17 \times 10^{-1}$	$3{\cdot}17 \times 10^{-2}$				
a_{gf}	2·02	$2{\cdot}14 \times 10^{-1}$				
a_{eu}	18·8	3·87				

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during the current study was considerably lower than this (mean $\pm$ S.E = 0·46 $\pm$ 0·055). This suggests that arsenic absorption in ruminants may be lower than in other animals, however further work would be required to confirm this. Although As(III) was administered during the current study, previous evidence would suggest that the absorption of As(V) is likely to be similar (Fowler *et al*. 1979). It has been suggested that inorganic arsenic can be methylated by rumen microflora (Lakso & Peoples 1975). There is also some evidence (from studies of non-ruminant animals) to suggest that absorption of some organic arsenic species is lower than that of inorganic arsenic (Fowler *et al*. 1979). It is thus possible that the comparatively low degree of absorption observed in the current study with sheep was due to organic arsenic, formed by methylation in the rumen, being less readily absorbed than inorganic species (as administered).

Maximum activity concentrations of ^{73}As were determined in the tissues obtained from the lambs slaughtered 0·5 days after administration; subsequently the loss of ^{73}As from all tissues was rapid (Figs 2 and 4). This rapid rate of decrease of arsenic in tissues is in agreement with earlier observations (Calvert & Smith 1981). However, it should be accepted that it is possible that peak activity concentrations in tissues occurred prior to the first sampling time (i.e. Day 0·5). Of the important tissues in terms of the human food chain, the initial ^{73}As activity concentrations increased in the order muscle $<$ liver $\approx$ kidney. Although some previous ruminant studies have suggested that liver is likely to contain less arsenic than kidney (Vos *et al*. 1988; Vreman *et al*. 1988), data for sheep reported by Calvert & Smith (1981) show a similar pattern to that observed in the current study. Skin, which was only sampled until 10 days after administration, appeared to be a potentially important pool of arsenic within sheep (Table 2). On the basis of this observation, it would have been preferable to sample this tissue for a prolonged period. However, the emphasis of the current study was human food-chain tissues (i.e. muscle, liver and kidney).

An improved fit to the data was achieved by dividing muscle, liver and kidney into two compartments (Table 3). There is some biological justification for this approach. As discussed earlier, inorganic arsenic may be methylated by rumen microflora (Lakso & Peoples 1975). Therefore, although As(III) was administered, arsenic entering the circulatory system could be a mixture of inorganic and organic arsenic depending upon the degree of methylation. Furthermore, a number of authors have demonstrated that inorganic arsenic is methylated to predominantly dimethylarsinic acid in the tissues of many non-ruminant species (Crecelius 1977; Tam *et al*. 1979*a*, *b*; Charbonneau *et al*. 1980; Goering *et al*. 1999; Healy *et al*. 1999). Lakso & Peoples (1975) have suggested that arsenic methylation also occurs in the tissues of ruminants. This enzymatic biotransformation occurs predominantly within the liver (Zakharyan & Aposhian 1999; Radabaugh & Aposhian 2000) although it is also thought to occur in other tissues (Walker & Griffin 1998). There is also limited evidence that As(III) is oxidized to As(V) (Bencko *et al*. 1976). These processes are thought to be detoxification mechanisms as As(V) and methylated arsenic compounds are less toxic than As(III) (Lakso & Peoples 1975; Bencko *et al*. 1976). The 'two compartment' pattern of loss of ^{73}As observed in muscle, liver and kidney during this study (see Fig. 4) could be due to the differing turnover rates of arsenic species in these tissues (Selby *et al*. 1974; Lakso & Peoples 1975; Crecelius 1977; Tam *et al*. 1979*a*). It is probable that this biological justification would apply equally to other pools. However, there were insufficient experimental data statistically to justify this extra complexity. The representation of the pools and pathways between them as presented in Fig. 3 may not be the most biologically accurate. For instance, as modelled, all arsenic entering the slow pools of liver, kidney or muscle must first be transferred back through the fast pool of that tissue before being excreted. If it is reasoned that the different pools within these tissues is the result of transformations of As(III) to other arsenic species, there is little evidence in the literature to suggest that back transformation of organic arsenic to inorganic arsenic occurs (Crecelius 1977; Fowler *et al*. 1979). However, it cannot be certain what each pool actually represents; only that, statistically, an improved description of the experimental data is obtained by division of those pools for which there are sufficient data. Other methods of representing the two pools for kidney, liver and muscle within the model structure were attempted but these did not further improve the fit to the data and some required the introduction of additional parameters.

The only time-series data available in the literature with which to test independently the dynamic predictions of the model is that of Calvert & Smith (1981), who present limited data for muscle, liver and kidney in a study involving the feeding of arsanilic acid (or *p*-aminophenylarsonate) incorporated into a pelleted grass feed for a period of 28 days after which two animals were slaughtered. Thereafter, an uncontaminated diet was fed for 6 days, during which time further animals were slaughtered at regular intervals. Details of the daily arsenic intake were not presented, only the concentration in feed. Therefore, a dry matter intake of 1·5 kg DM/day was assumed in the current study on the basis of the relationship between metabolic liveweight and the dry matter intake of pelleted feed presented in McDonald *et al*. (1995). Reported arsenic concentrations in tissues were presented on a dry matter basis; to make comparisons

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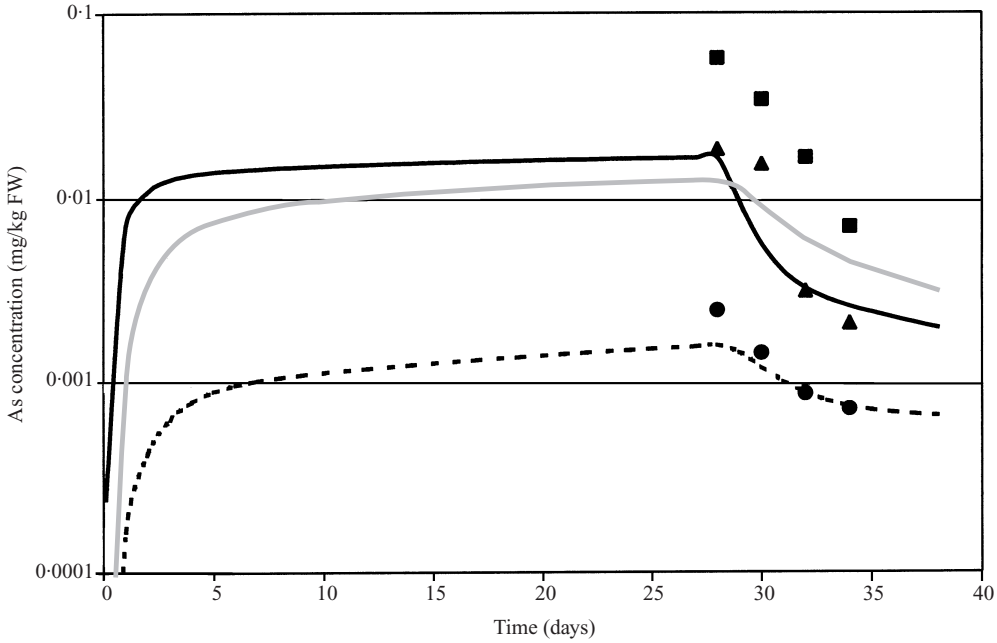


Fig. 5. A comparison of model predictions with the data presented by Calvert & Smith (1981). For comparative purposes, arsenic concentrations in feed have been normalized to 1 mg/kg DM and a daily feed intake of 1·5 kg DM has been assumed. ——, liver predicted; ——, kidney predicted; ----, muscle predicted; ■, liver observed; ▲, kidney observed; ●, muscle observed.

with model predictions these were converted to a fresh weight basis using tissue-specific moisture content values presented by Spectra (1956). For comparison with model predictions, experimental dietary intakes were normalized to 1 mg/kg. The resulting comparisons are presented in Fig. 5. Given that there is evidence to suggest that the rates of excretion of inorganic and organic arsenic species differ (Lakso & Peoples 1975; Crecelius 1977; Tam *et al.* 1979*a*), agreement between the model predictions and the observed data of Calvert & Smith (1981) may not have been expected. However, the level of agreement is encouraging, especially for kidney and muscle (Fig. 5). This suggests that although As(III) was administered the model can be used to predict the behaviour of organic arsenic species. Perhaps as a result of methylation of As(III) in the rumen and tissues experimental observations (and consequent model parameters) in the current study are partially reflecting the behaviour of organic arsenic. This reasoning assumes that arsanilic acid has similar turnover rates in tissues to methylated arsenic species.

Arsenic concentrations are predicted rapidly to reach equilibrium (< 40 days) with the dietary intake level (see Fig. 5). Therefore the use of equilibrium transfer coefficients (defined as the equilibrium ratio of the concentration of arsenic in a tissue (g/kg) to the daily dietary arsenic intake (g/day)) would seem valid when estimating food-chain transfer. The model has been used to estimate transfer coefficients for muscle, liver and kidney. The resultant values (± 1 S.E.) were $(2{\cdot}5 \pm 0{\cdot}67) \times 10^{-3}$ days/kg for muscle, $(9{\cdot}1 \pm 1{\cdot}96) \times 10^{-3}$ days/kg for liver and $(1{\cdot}1 \pm 0{\cdot}14) \times 10^{-2}$ days/kg for kidney. These values are in broad agreement with those recommended by Morgan (1991) on the basis of data presented by Calvert & Smith (1981) and van der Veen & Vreman (1986) of 2×10^{-3} days/kg (muscle), 5×10^{-2} days/kg (liver) and 3×10^{-2} days/kg (kidney) for a variety of organic and inorganic arsenic species.

Previous authors have implied that the potential for contamination of the human food chain with arsenic as a result of residues within tissues of animals exposed to arsenic is minimal as tissue concentrations will decline rapidly once the dietary source of arsenic has been removed (Calvert & Smith 1981). Whilst the results presented do demonstrate rapid turnover rates of arsenic within tissues relative to those of other heavy metal pollutants (Beresford *et al.* 1999), they also indicate a small fraction of arsenic in muscle with a slower rate of turnover (Fig. 4). This may have implications for the regulation of the entry of previously exposed animals into the human food chain especially as muscle represents the most important food tissue.

The model presented represents a considerable

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improvement in the ability to predict dynamically the contamination of ruminant-derived food products by arsenic. It is likely that ingested forms of arsenic could have a different bioavailability from that of the As(III) species employed in the current study. If the relative bioavailabilities could be determined, perhaps using *in vitro* techniques as suggested by Rodriguez & Basta (1999), then the rate coefficient describing absorption (a_{rg}) could be modified accordingly. A probable limitation of the current model is that whilst it may accurately predict transfer at comparatively low levels of arsenic intake, it may not do so at higher intake rates when absorption from the gut may be saturated (Gonzalez *et al*. 1995) or toxic effects may occur (Biswas *et al*. 1998).

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Note: Animal experiments were conducted under the auspices of the Animals (Scientific Procedures) Act 1986 and in accordance with 'Principles of laboratory animal care' (NIH publication no. 85-23, revised 1985). Furthermore, the experimental procedures were approved in advance by the Ethics Committee of the Macaulay Land Use Research Institute.

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