REVISION OF TRITIUM EXPERIMENTAL DATA FOR FARM ANIMALS

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COWS AND MINIGOATS

1) Radiation Protection Program. Final Report. Contract no. BI6-051-NL. Agricultural University, Wageningen, The Netherlands

Organically bound tritium (OBT) in the feedstuff administrated to cows and minigoats was obtained by spraying young growing grass with HTO. After drying, in order to evacuate HTO completely, the resulting hay had been administered to the experimental animals which had been equipped with fistulas in either rumen or abomasum (4^{th} stomach) or in both. The tritiated feed was administrated daily in doses of about 9.5 μCi (351 kBq) for varying periods of time, e.g. during the entire pregnancy period of 150 days and / or during the lactation period (80 – 220 days). The milk was monitored. In other series of experiments, the young animals were left with their mothers after birth during lactation period. OBT administration was discontinued at weaning, and animals were sacrificed at 0, 7, 21, 48, 92, 115, 155, 205, 240, 276 and 280 days, respectively.

Carbon-14 was incorporated into growing corn plants by exposing them to $^{14}CO_2$ for 8 hours. After plants harvest at the full-grown, the corn was cut, thoroughly mixed and fed to a lactating cow for a period of 33 days. The average daily activity ingested was about 105.6 μCi (391 kBq).

Tritium and carbon levels were determined in organic milk constituents (milk casein, milk fat and milk sugar or lactose), in milk water and in organs and tissues of young animals by combustion of samples and subsequent liquid scintillation counting.

There were measured the tritium levels in about 30 organs and tissues amounted to 5.05 pCi (187 Bq) and 4.80 pCi (178 Bq) per gram of dry matter, respectively with a standard deviation of about 17 %. The half-life values must be considered as averages. The decrease of tritium in the organic material of some organs and tissues could be described more satisfactorily by two components than in others. The values for the most representative organs are given in Table 1.

Table 1. Biological half-times (as averages) for different organs

Organ	**$T_{1/2}$ (days)**
Liver	14 – 81
Kidney	14 – 105
Large intestine	15 – 137
Skeletal muscle	128
Kidney fat	53
Subcutaneous tissue	78
Plasma	12

These fast components also have the largest pool sizes.

Determination of tritium levels in various tissues of young and adult animals indicate that important OBT deposits are located in body fat and in muscular tissue. Analysis by column chromatography had shown that, at steady state, more than 96 % of body fat deposits consisted of tritiated triglycerides. After about one year, tritium levels of body fat of adult animals decreased to 29 % of the levels at steady state, and to about 20 % in muscular tissue.

2) van den Hoek J, Gerber GB, Kirchmann R (1984) Transfer factors and turnover of THO and of organically bound tritium in two relate animal species with a view on modelling. The Environmental Transfer to Man of Radionuclides Released from Nuclear Installations. Commission of the European Community, Luxembourg

Two Friesian cows drank tritiated water with specific activity (SA) of about 19 nCi of tritium per ml for a period of 25 days. Grass was sprayed regularly with HTO under a plastic cover, store at full harvest and dried to hay. After evacuation of the remaining HTO by drying in a vacuum stove at 37 °C, the hay was administered through a rumen fistula to two other Friesian cows for a period of 26 and 28 days, respectively. Two miniature goats received tritiated hay also in the same manner during the entire gestation period of about 150 days and for 12 and 15 days, respectively during the lactation period. The cows and goats receiving tritiated hay were fed *ad libitum* with hay of excellent quality; they did not receive any concentrate or other supplements. Milk was sampled daily in all animals and milk water, milk fat, casein and lactose were separated by conventional methods.

The transfer of tritium from cow's diet to her milk of both HTO and OBT is about 1.5 % of the daily ingested tritium per litre of milk emphasizing that there is no important dilution by stable hydrogen from non-tritiated food or water. After ingestion of OBT, about 53 % of the tritium are in milk water and about 47 % in the organic milk constituents. Percentages from this organically bound fraction are found in the following components: 34 % in milk fat, 11.5 % in casein and only 2.5 % in lactose.

After ingestion of HTO, more than 96 % of the tritium in milk are found in the milk water.

There are no data for the incorporation of tritium in the organic milk constituents and in milk water of the goat after continuous ingestion of HTO, but such data have been collected after continuous administration of OBT. Results of this comparison between cows and goats were normalized for a daily intake of 1 mCi of OBT. The secretion and incorporation of the daily ingested OBT into organic milk constituents and into milk water is much higher in the goat than it is in the cow for the same tritium intake. The cow ingested about 21 times as much hay as did the goat, and this dilution appears to explain quite well the differences in ^{3}H incorporation into casein and lactose but not those for milk fat. At equilibrium, tritium activity in milk fat of cow is more than twice as high as in casein, whereas in the goat tritium levels in casein are higher than those in milk fat. The goat synthesizes nearly one half of the fatty acids herself against only 28 % by the cow.

Tritium content in milk water is higher for goat than for cow. This may be explained by the higher water turnover in the cow than in the goat. The combustion water in the cow will undergo a greater dilution in the body water pool than is in the case of goat.

In the case of OBT ingestion, the cow transfers about 1.6 % of the daily ingested OBT per litre of milk against about 37 % for the goat.

The distribution of tritium in organic milk constituents and in milk water of the goat after continuous ingestion of OBT is as follows: in one litre of milk, 75 % of the tritium are in milk water, 11 % are in milk fat, 11 % in casein and 2.5 % in lactose. The relative incorporation of tritium in casein and lactose is similar to that in cows, but for milk fat a much lower value is found. The fraction of tritium in milk water of the goat is higher than that in the cow.

The general behaviour of tritium in the organic milk constituents is similar in both species. There is a rapid decrease initially in all cases, followed by a much slower loss of tritium activity later. Tritium levels in casein are higher than in milk fat in both cow and goat and the decrease in lactose is rapid in both animals. After 100 days, tritium levels in casein of goat milk are about 7 % of those of steady state and those in milk fat about 2 – 3 %.

The decrease of tritium activity in different milk constituents of both cow and goat can be resolved into three components of rapid, intermediate and long half-lives, respectively. There is a good agreement between the half-life values of milk constituents of cow and goat except for those of the long component of casein and milk water, which are considerably higher in the goat. It is interesting that the pool or pools of slow turnover for casein precursors have a longer turnover time than those for milk fat precursors.

COWS

1) Potter GD, Vattuone GM, McIntyre DR (1972) Metabolism of tritiated water in the dairy cow. Health Phys 22:405–409

A lactating Holstein dairy cow from the LLL dairy herd weighing 530 kg and producing approximately 18.8 kg of milk per day was given 280 mCi (0.55 ml at 1.158×10^{12} dis/min per ml) of tritiated water orally. The cow was catheterized and maintained in a metabolic stall for the first 6 days of the experiment to facilitate complete collection of urine and faeces. The cow had free access to water and alfalfa hay and was given a supplement of 3 kg of dairy mix at each milking. The evening and morning collections were pooled and thoroughly mixed to provide 24-hr samples of milk, urine, and faeces. Blood samples were taken at 0, 3, 6, 12 and 24 hr and at each 24-hr interval thereafter. Plasma and cells from each 24-hr sample were separated by centrifugation for analysis of tritium during the first 144 hr (6 days) of the experiment. Additional spot samples of milk, urine, faeces and blood were collected up to 1704 hr (71 days) at suitable intervals. The experiment shows the mean value and the range for HTO in blood, milk, urine and faeces versus time. The loose body-water tritium is in equilibrium throughout the body-water pools. The curve for tritiated water had two components. The half-life of the fast component was 3.1 days. However, after 40 days a slow component with a half-life of about 33 days was also observed. The lose-water tritium in blood had equilibrated by 6 hr; its concentration in faeces did not reach a peak until the second or 48-hr collection.
Incorporation of organic tritium in the blood did not reach peak levels until 48 hr after administration, although the loose HTO in blood appeared to equilibrate within 6 hr. The two components representing the disappearance rates for bound tritium in blood have half-lives of 12.5 days and 65 days, respectively.
The dose is unique and approximately 1.2 % of the administered tritium in the cow would be organically bound.

2) van den Hoek J, Tenhave MHJ (1983) The metabolism of tritium and water in the lactating dairy cow. Health Phys 44:127–133

Two Friesian cows (cows A and C) were given tritiated water, SA of about 19 nCi of tritium per ml, to drink for a period of 25 days. Previous experiments had indicated that near steady state conditions for body water in the cow are reached after about three weeks. The tritium concentrations of the drinking water were kept as constant as possible during the time of HTO administration. Daily samples of milk and urine were collected. These cows in full lactation, on a diet with very low water content and with a daily water consumption of nearly 50l, secreted about 1.5 % of the daily ingested tritium in one litre of milk. For detailed results you have to see Excel Table. The average water content of the milk of the Friesian cow is 87 %. It follows from the data in Excel Table that more than 95 % of the tritium in milk are in the water phase so that the transfer of tritium from drinking water to milk is essentially a transfer from a water phase to another. Excel Table shows a value of 0.83±0.01 for the ratio of the specific activities for the two cows used in present study.
Near plateau levels in milk water were reached in both animals at about 19 days after the beginning of HTO ingestion, and the period of apparent steady state continued until day 26 when the administration of the HTO was stopped. A similar pattern of build-up of tritium, followed by a levelling off from day 19 onwards, can be observed

for the organic milk components. The fact that the value for milk water does not reach 100 % reflects dilution of the body water pool with non-tritiated water.
The dry matter of cow's milk has a relatively high content of lactose (4.6 %) and the incorporation of tritium in lactose is quite as high as shown by the ratio value of 0.58 for the specific activity of carbon-bound tritium in liver glycogen of mice.
Half-time can be calculated from the turnover time (T) divided by 1.33. When this is done for the period of tritium decrease, half times of 3.6, 4.1 and 4.3 days for the fast components of milk water, milk fat and casein, respectively are obtained. The longer lived components of the same labelled milk constituents have half-times of 43.7, 224.8 and 24.3 days, respectively. The slow components represent only about 4 % of the total milk tritium under conditions of infinite continuous administration of HTO and less than 0.1 % after a single dose.
The rapid decrease of tritium activity in milk water reflects the high rate of water turnover in cow. After a single dose of HTO the average value for the half time of body water was 3.54±0.10 days for lactating cows and 3.4±0.18 days for male bovines.

3) van den Hoek J, Tenhave MHJ, Gerber GB, Kirchmann R (1985) The transfer of tritium-labelled organic material from grass into cow milk. Radiat Res 103:105–113

Two Friesian cows (cow D and H) were kept in an experimental stable especially designed for carrying out experiments with radioisotopes on large animals. They were given tritiated hay by means of a rumen fistula for a period of 26 and 28 days, respectively. The animals were kept in the stable for about 7 months and were then allowed on pasture. Cow H was 3 months pregnant at the time of tritium administration. After the birth of the calf, milk sampling was continued during the second lactation period. Cow D did not become pregnant and therefore did not have a second lactation period. Cow H received about 10 times the daily dose of OBT given to cow D to allow the determination of tritium into milk constituents for a longer period after stopping tritium administration. To study the possible effect of dietary differences in stable hydrogen content, cow D received hay and concentrates and cow H hay only. As a result of this, cow H ingested about 75 % of the amount of stable hydrogen given to cow D.
Milk was sampled daily during the administration of organically bound tritium (OBT) and for about 1 month thereafter. Milk sampling became gradually more infrequent but was continued during the whole lactation period until the animal entered the "dry" period. In the case of cow H, it was resumed after the birth of her calf and continued during the entire 8-month lactation period, which followed.
The experiment shows the transfer of the ingested OBT to organic milk constituents, milk water, and whole milk for the entire sampling period of almost 450 days for cow H which received the high dose of OBT. The distribution of ^{3}H in milk components in cow D was quite similar, but the tritium levels were proportionally lower. Tritium could be detected easily in milk fat and casein during the entire second lactation period of cow H, that is, about 190 to 420 days after the feeding of OBT. However for lactose, milk water, and whole milk, the limits of detection were reached after about 260 days. The values and the fraction of ^{3}H in milk components derived from ingested OBT are given in Table 2, as well as the specific activity of tritium in milk components relative to that in the ingested hay.

Table 2. Tritium fractions in different milk components

Milk components	HTO (%)	OBT (%)	Relative specific activity
Fat	2	98	0.84
Casein	3	97	0.49
Lactose	39	61	0.05
Water	-	-	0.10

Expressed on a specific activity basis, tritium levels in casein, lactose, and milk water at steady state were 58, 10, and 11%, respectively, those of milk fat.

The high relative specific activity of 0.84 for milk fat is surprising. About 40 % of the fatty acids in bovine milk fat have chain lengths between 4 and 14 C atoms.

More specifically, the fraction of daily ingested OBT incorporated into the different components of 1 litre of milk is about 0.53 % for milk fat, 0.18 % for casein, 0.04 % for lactose, and 0.84 % for milk water. Quite different values are likely to be found in newly formed tissue which has lower water content than milk has (about 87 %) and different composition of organic material.

The first, short-lived component for milk fat, casein, and lactose very likely reflects the rapid turnover of precursors from the animal's food which are utilized for the synthesis of organic milk components in the mammary gland. The second component is likely to be composed of several pools, one or some of which may be located in the liver. The third component represents the pools of slow turnover which show average half-lives of about 1 month for lactose and about 3 month for milk fat and casein. It should be remember that equilibrium conditions in these pools were not obtained during 28-day period of feeding OBT.

It has been showed in many balance studies in cows on hay diet that about 60 – 70 % of the ingested dry matter of hay will be absorbed by the wall of the gastrointestinal tract.

In the experiment reported here, the amount of OBT in animal tissue, which is converted into HTO as a result of catabolic processes, can be calculated provided that sufficient time has elapsed since OBT administration. This would be the case in the second month of lactation of cow H after the birth of her calf when tritium levels in body water are about 1.5 pCi/ml. Assuming that the size of the body water pool of a cow in full lactation is about 65 % of her body weight and that she has a water turnover of 20 % of the pool per day, cow H would have a daily turnover of 73.6 litres of water. This water contained 110 nCi of ^3H, which may be assumed to have originated entirely from the animal's body reserves. The amount of tritium secreted into organic milk constituents can be calculated easily and amounts to about 4.74 nCi per day. Some organically bound tritium would be used for other metabolic processes, but excluding this unknown fraction from our considerations, about 115 nCi of ^3H were being liberated daily from the body stores and excreted at about 240 days after dosing. This is equivalent to about 0.03 % of the daily-ingested amount of organically bound tritium. It must be remembered that the administration of OBT lasted for only 28 days, a period, which was completely insufficient for equilibrium conditions to be obtained in pools of slow turnover with an average half-life of between 80 to 90 days.

4) Kirchmann R, Rémy J, Charles P, Koch G, van den Hoek J (1973) Distribution et incorporation du tritium dans les organes de ruminant, paper IAEA-SM-172/81, Environmental behaviour of radionuclides released in the nuclear industry, IAEA Vienna, pp 385–402

Besides the contamination of grass and fodder by tritiated water and incorporation of tritium into milk constituents, the authors have also investigated the distribution of tritium in the organs of ruminants contaminated in various ways. A cow was sacrificed 23 days after ingestion of 250 mCi of tritiated water in a single dose.
The various organs were removed and analyzed for the content of ^3H in the tissue water and in the organic matter.

Table 3. Distribution of tritium in different cow's organs

Organ	**HTO**		**OBT**		**SA dm/ SA H_2O**
	nCi/ml H_2O	**SA=nCi ^3H/g H**	**nCi/g dm**	**SA=nCi ^3H/g H**	
M. smt.	57	515	11	155	0.30
Eyes	64	578	8	101	0.17
Pancreas	63	570	14	167	0.29
Brain	66	592	11	126	0.21
Intestine	62	560	9	106	0.19
Liver	65	588	25	368	0.63
Kidney	65	582	19	255	0.44
Ovary	65	588	20	281	0.48
Bile	66	595	20	276	0.46

5) van den Hoek J, Kirchmann R (1971) Tritium secretion into cow's milk after administration of organically bound tritium and tritiated water. Symposium of Radioecology applied to the protection of man and his environment, Rome, 7-10 September 1971

A single dose of HTO was administered to lactating cows, 220 mCi being given orally to the first animal and 250 mCi being injected intravenously to the second one. Milk production was determined and daily milk samples were taken. About 250 m^2 of normal pasture was sprayed with 280 litres of water, containing about 2 mCi of ^3H per litre. Spraying was repeated ten times between May 25th and June 15th. The grass was cut one week after the last spraying and transformed into hay in the normal way. Dry matter content and activity of the hay were determined. The hay was fed to two lactating cows for about one week. The results are the following:

Table 4. Cow 33 – HTO single oral dose

Milk components	Maximum activity	$T_{1/2}$ (d) during the 1st week	Tranf. Coef. per milk litre (%)
Water	24	3	0.2
Fat	48	3.7	0.2×10^{-2}
Casein	48	4	0.4×10^{-3}
Lactose	-	-	0.4×10^{-2}
Dry matter	36	3.5	0.7×10^{-2}

Table 5. Cow 3 – HTO single intravenous dose

Milk components	Maximum activity	$T_{1/2}$ (d) during the 1st week	Tranf. Coef. per milk litre (%)
Water	12	3	0.2
Fat	40	3	0.2×10^{-2}
Casein	48	3.5	0.3×10^{-3}
Lactose	48	3	0.4×10^{-2}
Dry matter	24	3.5	0.6×10^{-2}

Table 6. Cows 27 and 39 - ^3H in organic form – 8 days

Milk components	Maximum activity	$T_{1/2}$ (d) during the 1st week	Tranf. Coef. per milk litre (%)
Water	192	5	0.5
Fat	144	2	0.3
Casein	144	2	0.1
Lactose	-	-	0.03
Dry matter	144	2	0.4

In the tritium study, single doses were administered whereas the organically bound tritium was fed to the animals over a period of 7 days. An important thing is that the tritiated hay had a dry matter content of about 87 %. This means that it contained about 13 % of the water activity of which amounted to less than 10 % of the total activity. Tritium from HTO in hay appears to be responsible for about 60 % of the water activity in milk, and for less than 2 % of the activity in the organic constituents in milk, excepting its much higher contribution to the activity of lactose. Maximum activity occurs most rapidly in milk water and roughly 24 hours in the organic material. The value of 3 days for milk water is in good agreement with the average half-life of 3.5 days for the body water.

6) Mullen AL, Moghissi AA, Wawerna JC, Mitchell BA, Bretthauer EW (1977) Tritium Retention by Cows and Steers and Transfer to Milk. Ecological research series, EPA-600/3-77-076, Environmental Monitoring and Support Laboratory, Las Vegas, Nevada, USA

The dairy animals selected for the study were either Holstein or Jersey cows 3 to 9 years of age, were in their 30th to 100th day of lactation, and were producing 20 to 31 kg of milk per day. This study was conducted as a series of three consecutive experiments. The milk transfer portion of the study utilized two groups of four lactating dairy cows (three Holstein and one Jersey) in each group. Each of the cows was given a single 100-mCi dose of tritiated water. The milk from this group was analyzed for tritium activity in the whole milk. The second group of dairy cows received 200 mCi of encapsulated tritiated water each. Milk from these cows was separated into milk, serum, butterfat, and protein fractions and the tritium activity of each fraction was determined.

Tritium in the whole milk of cows appears to decrease with 3.04±0.09-day half-life during the 20 days following oral administration of 100 mCi of tritiated water. The transfer of tritium to milk then changed to exhibit a slope with half-time of 11.1±2.58 days followed by a much longer half-time of > 120 days. For the second experiment we have: the initial half-times shown by the milk components indicate little difference between the milk serum and the butterfat with half-times of 2.93±0.14 days and 3.28±0.35 days, respectively. The protein fraction of the milk exhibited a considerably shorter half-time of 2.08±0.56 days. The longer half-time portion of the curves showed the normal variance of results with time and lower activity. The shortest half-time was indicated by the tritium activity in the serum portion of the milk. This was found to be 43.7±4.28 days. The incorporation of tritium into butterfat decreased with a longer half-time of 60.7±43.0 days and then increased during the latter part of the study.

MINIGOATS

1) Van den Hoek J (1986) Tritium metabolism in animals. Radiat Prot Dosim 16: 117–121

This experiment shows ^{3}H levels in various milk constituents of a miniature goat who received OBT in her food for a period of 160 days, the final 14 days of which she was lactating. The metabolic oxidation of OBT from food for the formation of ATP has resulted in quite significant ^{3}H levels in body water. There is also direct use of OBT from food for the synthesis of functional body constituents as shown by the high ^{3}H levels in milk fat and milk protein, both of which are a result of de novo synthesis. Finally, the continued incorporation of ^{3}H into milk fat and milk protein from about 2 months after discontinuation of OBT administration onwards illustrates that tritiated precursors from body reserves have been channeled to the general metabolic pool. It serves as an indirect evidence for the existence of tritiated body reserves.
The experiment shows the results of a regression analysis carried out on the ^{3}H levels in milk constituents from a cow and two goats, which ingested OBT in their food for different periods of time. Three components can be distinguished with very short, intermediate, and long half-lives, respectively. The values for milk protein and milk fat of the third component are in good agreement. The half-lives of this third component are not very long.
In another experiment, a miniature goat was fed OBT containing feed for about 220 days. Administration of OBT was discontinued 100 days before conception. The kid, who was born 260 days after the last dose of OBT, was sacrificed immediately after birth and the ^{3}H levels in various organs and tissues were determined. The results show the average ^{3}H levels in casein of the mother goat during the first 30 days of lactation. Tritium levels in kid tissues and in milk protein are quite similar, which indicates that they were derived from the same protein source. Tritium levels in fatty tissues of the kid are twice as high as those in protein and the highest tritium content is found in the metabolically active brown fat. The conclusion is that tritiated amino acids and fatty acids from body reserves of the mother have made an important contribution to the synthesis of proteins and fats of fetal tissues.
Milk samples were taken regularly during a 200 day lactation period subsequent to the birth of the kid. Large fluctuations in ^{3}H content, particularly in milk fat, can be noted during the first 60 – 80 days of lactation. The fluctuations of ^{3}H levels in milk fat are reflected in those in body water, which also show differences of a factor of two or more.
Tritium levels in milk protein were relatively stable in the second part of the lactation period and they decreased with a half-life of about 170 days. This is twice the value of the long component.
There is another experiment which shows the tritium concentrations in various organs and tissues of a miniature goat which was sacrificed 350 days after a 190 day period of feeding of OBT. Tritium levels in muscular tissue are about three times those found in several organs and twice those in brain tissue. Body fat was sampled at two locations and the tritium content based on 10 independent determinations, shows appreciable differences, indicating that the turnover rate in these two fat depots have been quite different.
The experiments show the average ^{3}H concentrations in different milk constituents of three miniature goats over a 10 – 14 days period of lactation. The animals had received equal daily doses of OBT during the previous six months and they were on

the same dietary regimen. There are no apparent reasons which could explain the differences in ^3H transfer from OBT from food into organic components synthesized by the animals.

2) Kirchmann R, Rémy J, Charles P, Koch G, van den Hoek J (1973) Distribution et incorporation du tritium dans les organes de ruminant, paper IAEA-SM-172/81, Environmental behaviour of radionuclides released in the nuclear industry, IAEA Vienna, pp 385–402

Besides the contamination of grass and fodder by tritiated water and incorporation of tritium into milk constituents, the authors have also investigated the distribution of tritium in the organs of ruminants contaminated in various ways. A pregnant goat was given water containing 46.6 μCi of ^3H/liter and the three kids were sacrificed at birth, at the age of one month or two months, respectively.
The results are given in Table 7.

Table 7. HTO and OBT concentrations in defferent organs

Organ	HTO (nCi/ml H_2O)	OBT (nCi/g dry matter)
Stomach	30.85	10.95
Tongue	31.14	9.68
Heart	31.16	10.38
Small intestine	30.79	9.70
Large intestine	31.04	10.00
Leg muscle	31.95	9.24
Dorsal muscle	31.80	10.09
Lungs	30.06	10.03
Liver	31.28	10.09
Brain	30.10	11.95
Kidneys	30.58	4.59

PIG AND PROGENY

1) Radiation Protection Program. Final Report. Contract no. BI6-051-NL. Agricultural University, Wageningen, The Netherlands

The metabolism of OBT and transfer of ^{3}H to the progeny has been studied in a pregnant sow given for 84 d before delivery and 42 d during the lactation period a tritiated diet consisting in 41% milk powder (636 Bq/g), 2.3% dried algal hydrolysate (3087 Bq/g), 51% dried potato powder (481 Bq/g) and 5.7% minerals. As pregnancy progressed and during lactation, the amount of food had to be increased correspondingly (1.86 kg/d from – 84 to – 63 d before delivery, 2.06 kg/d until – 38 d, 2.31 kg/d until – 5 d, 3.01 kg/d until day 15 of lactation and 3.66kg/d until day 41). In order to learn how much activity was transferred in uterus or during the lactation period and how this activity subsequently, 3 non-contaminated piglets from a control sow were transferred to the radioactive sow 3 days after delivery, 2 contaminated piglets were placed with non-radioactive sow and 3 contaminated piglets were left with their contaminated dam. Shortly after birth and 24 d later, at weaning and some time after terminating radioactive feeding, piglets were sacrificed, and OBT and tissue free water tritium (TFWT) activity were determined in different tissues. Measurements were also taken in urine and feces.
OBT activity was highest in erythrocytes, heart and kidneys, and relatively lower in bone, liver and muscle. For tissue free water tritium, the biological half-time is 5 d in the new born and 10 d in the weaned piglets. If changes in weight are taken into account, parenchymal organs, such as kidneys and liver, lose activity shortly after weaning at half-time of about 10 – 20 d and later more slowly at a half-time 20 – 50 d. Skin and muscles OBT has a half-time of 100 d and more.

2) Van Bruwaene R, Kirchmann R, Charles P, van den Hoek J (1976) Digestibility of cow's tritiated milk powder by calf and pig. International Symposium on Nuclear Techniques in Animal Production and Health as Related to the Soil-Plant System. Vienna (Austria), 2 Feb 1976. Report no. IAEA-SM-205/53, pp. 403-409

Milk obtained from a lactating cow maintained in a byre equipped for radionuclide experiments, fed on tritiated drinking water (226 µCi/l), was used in these experiments. Tritium passes into the various metabolic pathways that eventually produce milk. The cow was fed on 'cold' fodder in sufficient amounts for normal milk production. The yield of milk over a 40-day span was around 14 litres daily. A total of 419.3 litres of tritiated milk was dried to 51.343 kg of milk powder in spray-type machine. Daily milk samples were taken to allow determination of tritium activity in the following milk components: water fraction, milk fat, casein, albumin, lactose and whole milk dry matter.
In this experiment, three young pigs (about two months old), in metabolic cages, were given a mixture of flour and tritiated milk powder in increasing amounts. The rations conformed to the normal daily ration for fattening pigs. Samples of urine and faeces were taken. The animals were slaughtered after 14, 21 and 28 days, the total ingested activity being 60.6 µCi, 110.4 µCi and 154.5 µCi respectively. Samples of different organs were taken.

Table 8. Activities of different milk components

Milk component	Activity (nCi/g)	Component fraction (g component/ kg milk)	Total ^{3}H activity in dry matter (%)
Dry matter	57.2	940	100
Water	180	60	-
Fat	70	261	36.5
Casein	38.4	222	17
Albumin	37.7	46	3.4
Lactose	61.2	353	43.1

Table 9. Biological half-lives of the different milk components after a single oral dose of HTO

Milk constituents	$T_{1/2}$ (d) during the 1st week
Dry matter	3.5
Water	3
Fat	3.7
Casein	4
Lactose	-

Table 10. Results of digestibility of tritiated milk powder for pigs

Age (weeks)	6	6	6
Time of experiment (days)	14	21	28
Digestibility coefficient	0.923	0.924	0.908
Muscle			
Retention (kg of whole muscle) (%) (dm)	0.31	0.28	0.21
Retention (kg of newly formed muscle) (dm)	0.17	0.80	0.40
Liver			
Retention of ingested activity in whole liver (%)	3.02	2.83	2.84
Retention (kg of normal liver) (%)	0.83	0.79	0.79

3) Kirchmann R, Charles P, van Bruwaene R, Remy J, Koch G, Van den Hoek J (1978) Distribution of tritium in the different organs of calves and pigs after ingestion of various tritiated feeds. Curr Top Radiat Res Q 12:291-312

Each experiment was performed on male 7-weeks old pigs from the same litter, the first week being devoted to acclimatization in the metabolic cages. Pigs P2, P3, P4 were injected, intraperitoneally, with 10 ml of tritiated water (3.93 mCi/ml). Pigs P5, P6, P7 received daily 28.4 μCi of tritiated water (800ml HTO and 35.5 μCi/l). One animal (P7) died during the experiments. The total activity ingested as HTO was until slaughtering respectively 568 μCi (P5) and 766.8 μCi (P6). In a third experiment, three pigs (P8, P9, and P10) ingested daily 1,000 g of boiled tritiated potatoes (2.3 nCi/g of fresh matter, 1.3 nCi/g of dry matter and 0.6 nCi/g of starch). The ratio was further completed by the usual mixture. The total activity ingested by each animal, until slaughtering, was respectively 21 μCi (P8), 40.3 μCi (P9) and 48.1 μCi (P10). In a fourth experiment, three pigs (P11, P12, and P13) ingested daily tritiated milk powder of the same origin as the power used in the experiment with calves. The tritiated milk powder (51.2 nCi/g powder, 18.3 nCi/g fat, 8.5 nCi/g casein, 1.7 nCi/g albumin and 21.6 nC/g lactose) substituted milk powder of mixture. The total activity ingested by each animal, until slaughtering, was respectively 60.6 μCi (P13), 110.4 (P11) and 154.5 μCi (P12).

Table 11. Distribution of tritium in organic fractions of pig organs

Organs	**^{3}H in water**	**^{3}H in milk powder**	**^{3}H in potatoes**
Lungs	0.190	1.11	2.00
Liver	0.400	0.98	4.47
Spleen	0.045	0.16	0.36
Pancreas	0.031	0.23	0.32
Duodenum	0.086	0.86	1.86
Large intestine	0.190	4.20	2.78
Kidneys	0.056	0.47	0.80
Muscle	6.0	31.70	96.6
Brain	0.023	0.10	0.23
Thymus	0.019	0.27	0.36
Sum	**7.00**	**40.8**	**109.8**

The tritium content of pig urine injected with tritiated water decreases with a biological half-life of 3.8 days. This rate of total incorporation increases by a factor of 5.6 when ^{3}H is ingested by pigs as tritiated milk powder as compared to HTO and by a factor 15.6 when tritiated potatoes are ingested. The amount of ^{3}H fixed in the organs is 4.08% of ^{3}H ingested as tritiated milk powder were found in the organic fraction of pigs, which represents 49% of the whole animal.

4) Van Bruwaene R, Gerber GB, Kirchmann R, van den Hoek J, Vankerkom J (1982) Tritium metabolism in young pigs after exposure of the mothers to tritium oxide during pregnancy. Radiat Res 91:124-134

Two sows of the Belgische Land Ras strain were mated and given drinking water containing (A) 0.517 mCi/litre or (B) 1.531 mCi/litre of tritium oxide during 77 days of pregnancy. A third sow (C) was mated at the same time and kept as a control. The sows were fed a standard diet and had free access to water. The total amounts of water consumed by the contaminated sows and the weights of the animals are presented in Table 12.

Table 12. Total amount of water consumed by contaminated sows

Sow	Dose (mCi/L)	Weight (kg)	Water consumption after 77 days (L)	Water consumption after 120 days (L)
A	0.517	158	535	975
B	0.532	138	458	1022
C	Control	131	Not determined	Not determined

At delivery, three young pigs from sow A and two from sow B were transferred to control sow C, while six uncontaminated young pigs were placed with sows A and B (three with each sow). Application of tritium oxide was continued after delivery for 43 days. Young pigs from each group were sacrificed at 23, 43, or 115 days. Organs were removed and weighed, and the tritium activity in water and organic matter was determined.

When tritium oxide is given to pregnant sow, the activity of tritium in plasma water rapidly approaches equilibrium at relative specific activities of 0.662±0.042. Turnover time of 10 days but cannot be estimated exactly since activity was determined from day 29 of pregnancy when equilibrium had nearly been attained. Equilibrium in serum proteins requires more time (T_0=40 days) and occurs at specific activity ratio of 0.095±0.005. No significant difference in this behaviour is discernible between the two sows given the two doses of tritiated water. In newborn pigs, the specific activity ratio of tritium oxide is the same in all organs independent of the dose, but newborn from the sow on the low dose transferred to an uncontaminated mother lost tritium activity somewhat faster than the newborn from the mother on the high dose. Uncontaminated young pigs transferred to a mother drinking tritium oxide rapidly approach a constant specific activity ratio in their tritiated water pool.

Contaminated young pigs maintained with their mother and receiving tritium oxide until 43 days after birth display a slight change in tritium oxide relative specific activity from 23 days and a rapid fall when tritium oxide application is ended at 43 days. Turnover of tritium oxide in adult pigs is nearly equivalent to that in other species. It may be somewhat more rapid in newborn pigs (mean 13 days). In young pigs and most likely also in adults, a second small (< 20%) metabolic component of tritiated water with a longer turnover time is apparent, but cannot be defined exactly due to the paucity of data for longer times. OBT attains an equilibrium value of about 14% of that of the tritium oxide consumed. Only brain has a significantly greater activity (about 23%). Turnover of organic tritium varies among organs; it is slowest in brain, faster in muscular tissue, and most rapid in liver and intestine. Serum proteins have about the same turnover as organs and the same equilibrium value.

5) van Hees M, Gerber GB, Kirchmann R, Vankerkom JH, Van Bruwaene R, Retention in Young Pigs of Organically Bound tritium given During Pregnancy and Lactation (1986) Radiat Prot Dosim 16:123-126

A pregnant sow of the Belgishe Landras strain, weighing about 180 kg, was given organically bound tritium (OBT) at an average dose of 15.6 nCi per g of food for 84 days before delivery and 42 days during lactation. The food consisted of a mixture of OBT contained in dried algae, potato, and milk powder. As pregnancy progressed and during lactation, the amount of food had be increased correspondingly (1.86 kg per day from – 84 to – 63 days before delivery, 2.06 kg until – 38 days, 2.31 kg until – 5 days, 3.01 kg until day 15 of lactation and 3.66 kg until day 41). Three days after

delivery, three non-contaminated piglets from a normal saw, two contaminated piglets were placed with the non-radioactive sow and the three contaminated piglets were left with the contaminated sow. Shortly after birth, at weaning and some time after terminating radioactive feeding, piglets were sacrificed, and OBT and tritium oxide activity was determined in different tissues. Radioactivity was also measured in urine and feaces. The sow was sacrificed 80 days after terminating tritium exposure and was assayed for tritium activity in tissue.
The results are given in the following tables:

Table13. Specific activity (Bq*g per 10^3 Bq* g^{-1} food) given at different ages of young piglets removed two days after birth from their contaminated mother.

Age (days)	**1**	**24**	**42**
Weight (kg)	1.423	5.568	11.8
Tritium oxide	195	nd*	nd*
Organic tritium			
Erythrocytes	1307	286	152
Liver	626	73	21
Kidney	1178	102	33
Muscle	641	135	69
Heart	1200	152	58
Skin	840	98	27
Bone	634	111	67
Brain	924	351	188
Fat	nm#	30	nd*

* not detected
\# not measured

Table 14. Specific activity (Bq g^{-1} / 10^3 Bq g^{-1} food) at different ages of young piglets kept during suckling (until day 40) with their contaminated mother.

Age (days)	**1**	**40**	**61**	**145**
Weight (kg)	1.269	11.9	24.3	101
Organic tritium				
Tritium oxide	194	270	2.9	nd
Erythrocytes	1307	1342	530	54
Liver	626	1036	209	20
Kidney	1171	1201	265	25
Muscle	641	1040	668	69
Heart	1200	1090	419	42
Skin	840	845	463	80
Bone	634	844	278	23
Brain	921	935	574	127
Fat	nm	nm	nm	29

Table 15. Specific activity (Bq g^{-1} /10^3 Bq g^{-1}) at different ages of young piglets placed at an age of 2 days with a contaminated mother until weaning at 43 days.

Age (days)	**43**	**64**	**148**
Weight (kg)	11.1	23	84
Tritium oxide	236	13	nd
Organic tritium			
Erythrocytes	1220	442	43
Liver	798	105	29
Kidney	982	179	37
Muscle	791	342	81
Heart	1045	290	45
Skin	842	342	104
Bone	937	305	43
Brain	744	363	100
Fat	882	340	75

GENERALITIES

1) van den Hoek J, Kirchmann R, Juan NB (1979) Transfer and Incorporation of Tritium in Mammals. Proc. Symp. Behaviour of Tritium in the Environment, pp. 433-444. San Francisco, California, USA

Single doses of tritium may be administered either orally or parentally. This means ingestion via drinking water in the former case, and intravenous, intramuscular or intraperitoneal application in the latter. Single dose experiments have been carried out on domestic animals in all participating laboratories.

Table 16. Biological half-life (days) for different species

Specie	Biological half-life (days)	
	1st component	2nd component
Mouse	1.1	114±50
Kangaroo rat	13.4±0.7; 13.2±1.3	
Goat (lactating)	4.1±0.1(2.9-5.3)	
Goat (non-lactating)	8.3±0.5(6.7-10.4)	
Mini goat (non-lactating)	4.3±0.2	
Pig	3.8-4.3	
Cow (lactating)	3.1; 3.3; 3.5; 4.0	33
Cow (non-lactating)	4.0±0.2	40
Chicken	4.6	

CALVES

1) Van Bruwaene R, Kirchmann R, Charles P, van den Hoek J (1976) Digestibility of cow's tritiated milk powder by calf and pig. International Symposium on Nuclear Techniques in Animal Production and Health as Related to the Soil-Plant System. Vienna (Austria), 2 Feb 1976. Report no. IAEA-SM-205/53, pp. 403-409

The milk powder was fed to two calves in metabolic cages. Milk obtained from a lactating cow maintained in a byre equipped for radionuclide experiments, fed on tritiated drinking water (226 μCi/l), was used in these experiments. Tritium passes into the various metabolic pathways that eventually produce milk. The cow was fed on 'cold' fodder in sufficient amounts for normal milk production. The yield of milk over a 40-day span was around 14 litres daily. A total of 419.3 litres of tritiated milk was dried to 51.343 kg of milk powder in spray-type machine. Daily milk samples were taken to allow determination of tritium activity in the following milk components: water fraction, milk fat, casein, albumin, lactose and whole milk dry matter. Each received twice daily a ration composed of 125 g of milk powder diluted in one litre of demineralised water and three litres of 'cold' fresh milk. Samples of urine and faeces were taken daily. After 28 days, each calf has ingested a total of 482.5 μCi, and was slaughtered. Samples of different organs were taken.

Table 17. Distribution of tritium in the milk powder components

Milk component	Activity (nCi/g)	Component fraction (g component/ kg milk powder)	Total ^{3}H activity in dry matter (%)
Dry matter	57.2	940	100
Water	180	60	-
Fat	70	261	36.5
Casein	38.4	222	17
Albumin	37.7	46	3.4
Lactose	61.2	353	43.1

Table 18. Biological half-lives of the different milk components after a single oral dose of HTO

Milk constituents	$T_{1/2}$ (d) during the 1st week
Dry matter	3.5
Water	3
Fat	3.7
Casein	4
Lactose	-

Table 19. Results of digestibility of tritiated milk powder for calves

Age (days)	**18**	**40**
Time of experiment (days)	28	28
Digestibility coefficient	0.989	-
Muscle		
Retention (kg of whole muscle) (%) (dm)	0.11	0.13
Retention (kg of newly formed muscle) (dm)	0.40	0.56
Liver		
Retention of ingested activity in whole liver (%)	3.1	4.0
Retention (kg of normal liver) (%)	0.28	0.36

2) Kirchmann R, Rémy J, Charles P, Koch G, van den Hoek J (1973) Distribution et incorporation du tritium dans les organes de ruminant, paper IAEA-SM-172/81, Environmental behaviour of radionuclides released in the nuclear industry, IAEA Vienna, pp 385–402

Besides the contamination of grass and fodder by tritiated water and incorporation of tritium into milk constituents, the authors have also investigated the distribution of tritium in the organs of ruminants contaminated in various ways. Three young male calves ingested 1 mCi of tritiated water daily for 25, 33 and 40 days respectively and were then sacrificed; three other calves ingested tritiated milk; a calf whose mother had received about 11 mCi THO per day for 40 days during gestation received mother's milk for 3 ½ months and was then sacrificed.

Table 20. Repartition of tritium in different residues and organs of the calves

Repartition	**Tritium in various forms**			
	Water (calves I, II, III)		**Powdered milk (calves IV, V)**	
	OBT	**HTO**	**OBT**	**HTO**
Residues				
Urine	584.8		498.0	
Faeces	0.3	6.6	4.7	3.7
Organs				
Kidney	0.033	0.70	0.62	0.69
Bile	0.020	0.42	0.33	0.65
Brain	0.023	0.51	0.34	0.44
Lungs	0.051	1.15	0.78	1.90
Muscles	2.46	50.29	35.6	88.0
Tongue	0.030	0.59	0.43	0.85
Heart	0.046	0.72	0.90	1.02
Liver	0.14	2.55	3.44	3.50
Total organs	2.803	56.93	42.44	97.05

3) Kirchmann R, Charles P, van Bruwaene R, Remy J, Koch G, Van den Hoek J (1978) Distribution of tritium in the different organs of calves and pigs after ingestion of various tritiated feeds. Curr Top Radiat Res Q 12:291-312

Three male 10-day old calves, received twice daily a known volume of fresh milk, supplemented with 10 ml of tritiated water (50 μCi/ml). The total activity ingested until slaughtering was respectively: 25.6 mCi (calf I), 33.5 mCi (calf II) and 40.4 mCi of tritium (calf III). Two other male calves, respectively 18 and 40 days old on the first day of the experiment, received each, twice daily, 125 g of tritiated milk powder, diluted in one liter of demineralized water, the ration was completed with 31 of normal fresh milk. The total activity ingested during the 28 days experiment was 482 μCi per calf.

Table 21. Distribution of tritium in organic fractions of calf organs of total activity ingested

Organs	^{3}H in water	^{3}H in milk powder
Kidney	0.033	0.62
Spleen	0.020	0.33
Brain	0.023	0.34
Lungs	0.051	0.78
Muscles	2.46	35.6
Tongue	0.030	0.43
Heart	0.046	0.90
Liver	0.14	3.44
Sum	**2.803**	**42.44**

In the case of a daily ingestion of HTO by calves, only 0.28% of the ^{3}H ingested is found organically bound at the time of slaughtering, while if ^{3}H is ingested as tritiated milk powder, the rate of incorporation is 15 times higher. In calves 4.2% of ^{3}H ingested as milk powder are found in the dry matter of organs which constitutes 44% of the weight of the animal.

4) Mullen AL, Moghissi AA, Wawerna JC, Mitchell BA, Bretthauer EW (1977) Tritium Retention by Cows and Steers and Transfer to Milk. Ecological research series, EPA-600/3-77-076, Environmental Monitoring and Support Laboratory, Las Vegas, Nevada, USA

The beef animals were 4- to 6- year-old Hereford steers. All the animals were allowed free access to water and alfalfa hay. In order to determine the difference in blood tritium levels between lactating animals, three Hereford steers were each administered 65 mCi of tritiated water in the same manner as the dairy cows. Blood was collected from all three groups of cows and the serum portion analyzed by direct liquid scintillation counting. The tritium levels in blood serum from beef cattle were averaged and the half-lives determined to be 4.05±0.21 days and 40.36±9.82 days.