Measuring Transfer of ^{14}C-PCB from Maternal Diet to Milk in a Goat Model Using an Accelerator Mass Spectrometer (AMS)

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ABSTRACT

Environmental pollutants pose a substantial risk to nursing infants. Many of these toxicants (i.e., PCBs, PBDEs, mercury) are passed from the maternal diet to the nursing infant in breast milk. Determining the toxicokinetics involving the GI absorption and excretion has been difficult to measure in humans due to ethical limitations. However, newer technologies that enable measurement of very low levels of radioisotopes and the development of superior animal models are helping us to predict the relationship between maternal dietary intake and secretion in milk. Understanding the toxicokinetics will allow us for improved risk assessments which are necessary to protect the nursing infant from exposure to environmental pollutants.

A goat model was developed and a ^{14}C-labeled PCB (2,2',4,4',5,5'-hexachlorobiphenyl-UL-^{14}C) was used as a tracer. Use of a labeled compound makes it possible to track the absorption distribution and elimination of the compound without interference from background levels that are present from environmental exposure. A one-year-old, 45 Kg lactating Nubian doe who had kidded 2.5 months previously was milked twice a day to maintain lactation. Baseline concentrations of ^{14}C in milk was measured for 3 d. The goat was dosed orally with 0.084 mg of ^{14}C-labeled PCBs. The dose contained 3 µCi (dosage = 66 nCi/Kg). Blood and milk samples were collected for 2 months after dosing. The ^{14}C/^{12}C (10-15) ratio in the milk samples reached a peak value of 1.69 x 10^5 on the second day after dosing and then declined to about 1 x 10^4 ~3 wk after dosing and remained fairly stable until the end of the 2 month sampling period. The ^{14}C/^{12}C (10-15) ratio in plasma samples rose slowly and reached a peak value of 1.930E^{+04} at 24 hr. Plasma counts declined slowly until the third week where plasma and milk counts were approximately the same.

Our results indicated that it would be possible to use a lower dose of labeled PCB due to the extreme sensitivity of AMS measurement. Thus, the potential exists for developing protocols for studying toxicokinetics in humans using radiologically- and toxicologically-benign doses of environmental toxins.

INTRODUCTION

Studies of environmental contaminants in human breast milk have been conducted by obtaining samples of milk from lactating women and measuring the contaminant. These concentrations are then compared to estimated dietary intakes. Until recently, it has not been possible to measure the pharmacokinetics and distribution into milk following maternal ingestion of environmental contaminants. Accelerator Mass Spectrometry (AMS) is an ultra-sensitive technique which makes it possible to measure atto-mole quantities of compounds. The sensitivity of this technique will make it possible to give benign doses of radio-labeled contaminants to humans for measuring bioavailability and excretion.

Preliminary studies, using ^{14}C-PCB in a goat model were done to determine the minimum dose necessary to measure pharmacokinetics and track secretion in milk.

METHODS

A one-year-old, 45 Kg lactating Nubian doe who had kidded 2.5 months previously was used. The goat was milked twice a day to maintain lactation. Baseline milk samples were collected for 3 days. A ^{14}C-labeled PCB (2,2',4,4',5,5'-hexachlorobiphenyl-UL-^{14}C, $C_{12}H_4Cl_6$) was used as a model compound. The goat was dosed orally with 0.084 mg of labeled PCB. This was a dose of 3 µCi of ^{14}C (66 nCi/Kg). The PCB was placed in a gel capsule and administered orally. Blood samples were collected before PCB administration and hourly for 12 hr and at 24 hr and twice weekly thereafter for 1 month. Plasma was separated and sent to the AMS facility for analysis. Milk was collected twice a day for 2 months after dosing and samples were sent to the AMS facility for analysis.

The goat was allowed to dry out and was bred again. Milk collection for the second kid was begun 223 d after the dosing with the labeled-PCB. Blood was also collected from the kid.

Milk, serum and plasma samples to be measured on the accelerator were dried by vacuum centrifugation. Combustion and reduction procedures follow those developed at CAMS (Vogel 1992), with a few minor modifications. The graphite was placed in the ion source (Jackson, Elmore et al. 2004) which generates ^{13}C-currents of 500-750 nA with the corresponding $^{14}C_4$+ ion detection rates of about 200 Hz for a sample ^{14}C enrichment of about 2 x 10^{-12} of total carbon.

RESULTS

After maternal oral dosing with ^{14}C-PCB, the concentration in maternal plasma rose rapidly during the first 12 hr and reached a maximum at 24 hr. Figure 1 shows levels of ^{14}C-label in the blood and milk samples. Approximately 12% of the label was recovered in the milk during 3 weeks and levels declined by 14% over the next week. In milk, the counts rose to a maximum at 2 days following dosing and declined by 66% during the first week.

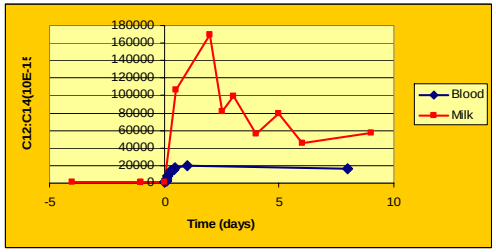

Figure 1. ^{14}C:^{12}C ratio in blood and milk.

Figure 2 shows the counts in the milk for the first and second lactation period. Counts in the milk declined rapidly over the first month and then continued to decline slowly during the first lactation period. The label was still detectable at a constant low level during the second lactation period 8 months after dosing.

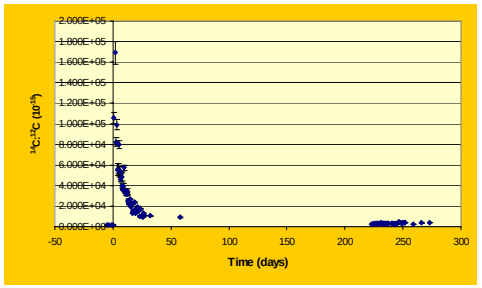

Figure 2. Counts in milk.

Label was detected in the plasma of the kid born 7.5 months after dosing. The ^{14}C:^{12}C (10^{-15}) ratio was 2.247 x 10^3.

CONCLUSIONS

- After oral dosing, PCBs are absorbed slowly and reach a peak in plasma after 24 hr.
- After oral dosing, PCBs reach peak levels in milk at 2 days and decline over the next 30 d.
- After 30 days, the concentration in milk declines slowly.
- PCB residues remain in the mother for at least 9 months and can be detected in subsequent lactations.
- AMS is a useful tool for the investigation of the transfer of toxic substances from the mother into milk. The sensitivity of the method allows toxicologically- and radiologically-benign amounts to be used as tracers.
- The dose of 66 nCi/Kg PCB that was used in this study was more than adequate and suggests that a smaller dose may be used in future studies.

REFERENCES

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