Purpose: To assess the importance of tissue segmentation for Monte Carlo (MC) dose calculations with kilovoltage photon beams and to suggest a new segmentation method based on effective atomic number differences.

Methods: MC dose to 34 ICRU-44 tissues was calculated in a phantom irradiated by a number of kilovoltage beams using the EGSnrc/BEAMnrc and DOSXYZnrc codes. Photon beams currently used in small animal radiotherapy were studied: a microCT 120 kV beam, two 225 kV beams filtered with either 4 mm of Al or 0.5 mm of Cu , a heavily filtered 320 kV beam, and a 192Ir beam. MC dose was calculated for a five 120 kV beam treatment plan for a mouse with lung cancer. The microCT images were segmented using three different segmentation schemes - with 4 (the DOSXYZnrc default), 8 , and 39 tissue types. The 4 -tissue and 8 -tissue segmentation schemes were based only on mass density differences. The new advanced 39tissue segmentation scheme is based on the combination of both mass density differences and atomic number differences.

Results: Using our model we showed that mis-assignment of adipose to cartilage caused dose calculation differences of $36 \%, 24 \%$, and $14 \%$ for the 120 kV beam and the 225 kV beams filtered with 4 mm Al and 0.5 mm Cu , respectively. A second order polynomial approximated well the absorbed dose to tissue as a function of the effective number for all beams. The small animal study showed that the doses to adipose and bone were overestimated by $30-50 \%$ when the 4 -tissue segmentation was used compared to the 8 -tissue and 39 -tissue segmentation schemes. The dose to the lungs and bone were underestimated by $5-10 \%$ with the 39 -tissue segmentation compared to the 8 -tissue segmentation.

Conclusions: A new tissue segmentation method was proposed for a more accurate MC dose calculations with kilovoltage beams.

