X-ray dosimetry for *in-vivo* microCT and radiation effects

Method note
MCT-143

1. Introduction

MicroCT achieves non-destructive 3D imaging using X-ray transmission and absorption through the images material – or animal. For *in-vivo* microCT imaging this involves an absorbed dose of X-rays to the mouse, rat or other scanned animal. This is unavoidable – X-ray absorption provides the essential data for the imaging process. The microCT dataset is simply a 3D map of X-ray absorption. This MN is about X-ray absorbed dose and what it means for *in vivo* microCT scanning.

"The dose is the toxin" is what toxicologists say about potentially harmful biological agents. Ionising radiation comes under toxicology since – at sufficiently high doses – it has a harmful effect on organisms, tissues, cells and chromatin within the nucleus of cells. If all ionising radiation was unacceptably harmful then *in vivo* microCT would not be possible, at all. But this is never the case for any toxin – there is always a safe dose and a toxic dose. Many chemicals routinely consumed safely such as caffeine in coffee are harmless at low doses but there exist a threshold above which the dose becomes toxic, even fatal. The same for ionising radiation. Every second that you have been reading this article about 10,000 radioactive disintegrations have occurred within your body. A similar number of high energy gamma photons have passed through you, originating in soil, building materials and cosmic rays.

So how does the question of X-ray dose relate to Bruker's high performance *in vivo* microCT scanners, the SkyScan 1276 and 1278? What is a harmful dose of ionising radiation? What doses are given during *in vivo* scans, and how do we measure them?

1.1. The SkyScan 1276 and 1278 in vivo microCT scaners

The Bruker SkyScan 1276 and 1278 *in vivo* scanners are of the rotating gantry type, where the X-ray source and camera are linked in a gantry and rotate around a cylinder horizontal central bore containing the sample bed. The same arrangement as in a hospital clincial CT scanner. However whether a scanner is of this *in vivo* rotating gantry type, or whether it is an *"ex vivo"* scanner in which

the source and X-ray camera are static and the scanned object rotates between them on a stage around a vertical axis, doesn't really matter regarding X-ray dose. What determines the dose received by the sample of animal is the same: it is the distance source-to-sample, the power and photon energy of the X-rays, and the time duration of the irradiation. How this dose is quantified is described a litle later in section 3.

1.2. X-ray source collimation

The X-ray source in both the SkyScan 1276 and 1278 scanners is collimated. (The same is true of the previous SkyScan 1176, 1178 and 1076 *in vivo* scanners.) This means that the X-rays are emitted through a thick metal window or "collimator" that is accurately positioned, so that only a few millimeters above and below the imaged field of view, the X-ray dose rate declines to zero (figure 1). X-rays outside the FOV are blocked. This is important, because it means that only the imaged part of the animal receives X-rays, plus a narrow bordering region of 1-2mm only.

It also means that in oversize scans, such as in the multi-part scan of a whole mouse, the dose received by the animal is equal to the dose of just one scan part, not the summed total of all the scan parts. There is a narrow region of overlap between neighboring oversize scans, where the mouse will be irradiated by two scans. Conservatively, this can be estimated to increase the total dose to the animal by about 15-20% more than the dose associated with a single scan part.

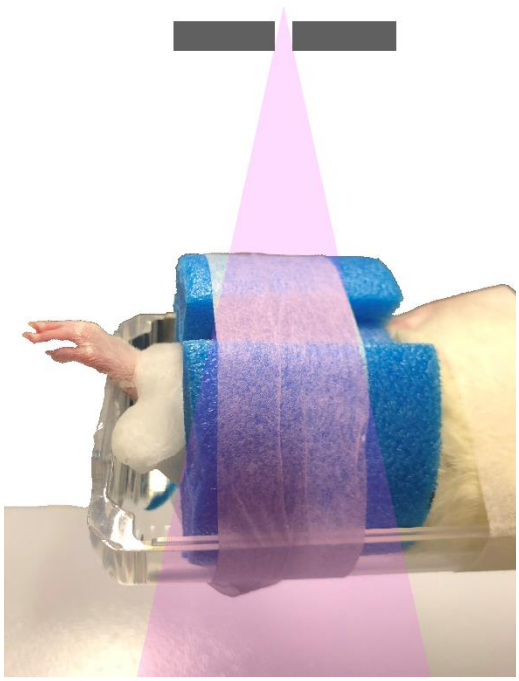


Figure 1. The X-ray source in the SkyScan1276 is collimated such that there is very little irradiation beyond the length (axially) of the camera field of view.

1.3. Zoom adds to scan flexibility in the SkyScan 1276 but also to X-ray dose

Image "zoom" – that is, variable magnification by altering the source-sample distance, adds considerably to flexibility in micro-CT imaging. It is a unique feature of the SkyScan 1276 *in vivo* scanner. However please note that zooming in sharply increases ionizing radiation dose. Zooming in bringing the source closer to the sample, and this increases dose by the inverse square of the distance. So for *in vivo* scans only a limited range of zooming is advisable in most cases. To keep X-ray dose to a minimum it is better to keep magnification at or near the minimum magnification, with limited zoom-in only. For instance at maximum (4k) camera resolution, from a (minimum zoom) 10 micron pixel down to about 7 microns. Higher zoom magnification is acceptable only if the scans are very fast, without excessive X-ray energy and power, and doses calculated to be in an acceptable range.

For *ex vivo* scans radiation dose is less important so zooming in can be done freely, the only restriction being the geometry of the scanner and sample. So for the SkyScan 1276, zooming (changing magnification continuously) provides a little flexibility for *in vivo* scans but can be used to its full extent in *ex vivo* scanning, where the scanner can provide a useful high resolution microCT imaging capability.

1.4. What does "low dose" mean in an in vivo scanner?

A low dose capability means being able to make a micro-CT scan of adequate image quality, with an ionizing X-ray dose that is low enough not to cause biological effects that would adversely affect the scanned animal and influence the biomedical study.

At mention of "low-dose" micro-CT *in vivo* scanning, a reaction of cynicism is understandable. Many *in vivo* microCT manufacturers claim that they are the one and only "low dose" solution provider. Some clarification is needed of what a "low dose" capability means. It is trivial and meaningless to claim that a scanner can scan a mouse at a low dose, since all that is needed for this is to turn the X-ray power to a very low level – as low as instrumentally possible. Use only a single microamp of source current for instance and any scanner is a low dose scanner.

The argument could be extended *"ad absurdam"* to just turning the source off altogether and scanning the animal with no X-ray exposure – the "zero dose scanner!" (patent pending…)

But the problem with just turning to very low or zero source power is that the image quality would be either extremely poor and noisy or there would be no image. So to have a meaningful low dose capability the scanner *must achieve an acceptable and useful image quality* with a low absorbed X-ray dose. So what is important is not just low dose *per se*, but low dose with useful image quality at the same time. In figure 2 below coronal microCT images are shown from *in vivo* scans of a mouse at doses of about 3 and 25mGy, showing that at the same dose level, different scanner models give image results with very different image quality.

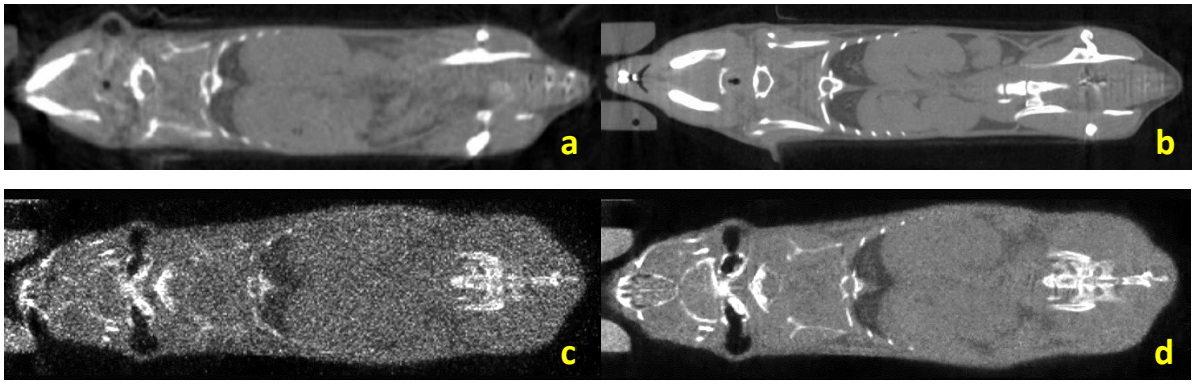
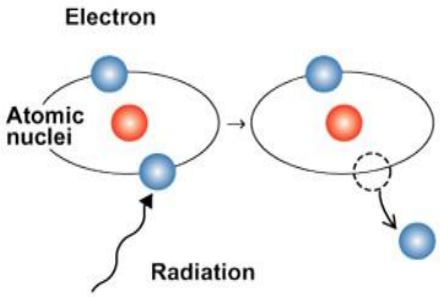


Figure 2. Coronal images of mouse scans, (a) by the SkyScan 1278 at 2.6 mGy and (b) at 25.8 mGy; and by a competitor scanner at 2.8 mGy (c) and at 37 mGy (d). The paired by dose comparisons of a-c and b-d show that at the same dose, the SkyScan 1278 image is of higher quality with superior signal to noise ratio.

2. A brief explanation of ionizing radiation and the radiation "dose"



What is ionizing radiation and radiation dose? X-rays are high energy electromagnetic radiation whose photons have high enough energy to dislodge electrons from atoms. When you kick out an electron from an atom, you change the electric charge and the chemistry of that atom or molecule by changing the charge balance between positively charged protons in the nucleus and negatively charged electrons. This is called ionization because a molecule or atom given electrical charge by change to its number of electrons, is called an ion.

Because electrons removed by ionization by X-rays are not removed by standard chemical reactions, this can result in unusual chemical species being created that normally don't arise from chemical interaction. These strange entities are called "free radicals". They include abnormally ionized species of organic molecules, hydroxide, hydrogen and oxygen. Free radicals are highly reactive, and dangerous and destructive in organisms and cells – like a molecular "bull in a china shop". A large part of ionizing damage to biological tissue including 60-70% of damage to genetic DNA is caused by hydroxyl free radicals ($OH^{\cdot}$)[1].

So most damage to animal tissues from X-ray in micro-CT imaging is caused by the creation of hydroxyl free radicals in the vicinity of chromosomes in the cell nuclei. What are the effects to be expected from X-ray radiation at different levels? To answer this, we first need a definition of the amount of X-ray irradiation of tissue. This is the "dose". Ionizing radiation dose is defined in terms of the physics of the ionization process. When X-ray photons ionize material, they transfer some of their photon energy to the absorbing material during the ionizing events. So radiation dose is defined as the amount of energy "deposited" in matter from the photons by these ionizing interactions. For a photon to dislodge a single electron from air requires energy of about 33eV (electron volts) which is very small, only 5.287 E-18 joules. The "joule" is the physical basic unit of energy, equal to 4.2 calories, the older unit of

energy (one calorie makes 1 cm^3 of water one degree C warmer) but still the unit you generally see on labels of jars of jam and chocolate spread for example. Many billions of such X-ray interactions with matter results in energy deposition by ionization that is measured in joules per kg. The name for one joule per kg transferred by ionizing radiation is the "Gray" after Louis Harold Gray, one of the early radiation scientists.

One Gray (or "Gy" for short) is a lot of radiation. You don't want to get a Gy, a whole body dose of 1 Gy can be fatal. Nor does your mouse in an *in vivo* micro-CT scanner. Thus discussion of X-ray ionizing dose is often in terms of milliGrays (mGy), one thousandth of a Gray. For convenience, hereon we will refer to X-ray ionizing absorbed dose as simply "absorbed dose" or just "dose".

$$Dose, Gy = \frac{absorbed\ energy, J}{mass, kg}$$

2.1. Dose is the concentration, not the amount, of absorbed ionizing energy

An important thing to understand about radiation dose is that it is a concentration, not a total amount, of ionizing energy. It's not an amount of energy in joules but a concentration in joules per kg. Misunderstandings about radiation effects can result from not understanding what the Gray actually is. For instance consider the following example. X-rays irradiate one foot of a mouse only. For example, the mouse is mounted with one leg in a polystyrene tube, and the purpose of the scan is to assess arthritic bone damage in the ankle. The dose that the foot receives is half a Gy from (say) a 3 minute scan. Another mouse receives a whole body scan, involving a 5-part oversize scan, each scan part being again 3 minutes duration. From this scan, the whole mouse is irradiated and so the animal receives a whole body dose of half a Gy.

Now in this example, the second mouse that gets a whole body scan clearly receives more radiation than the first mouse whose foot only is scanned. More ionization energy is deposited in the whole mouse than in the foot only. (As we'll mention below, the X-ray source is collimated so that only the imaged part receives X-rays. For this simple example we'll ignore overlapping irradiation at the boundaries between oversize scan parts.) But both animals receive the same dose, of 0.5 Gy. The unit of Gray means concentration of ionizing energy, not total amount, and shows no difference between these two scans. The whole body scan delivers more ionizing energy but it is distributed over more tissue mass. So the concentration of energy deposition is the same. The first mouse's foot gets 0.5 Gy, and the second mouse gets 0.5 Gy to its whole body.

So the Gray only tells us the local concentration of absorbed ionization. Another unit of radiation dose has been developed to give us an idea of the extent of irradiation over a person's body, and therefore how much radiation harm can be expected. Note that this "radiation harm" is mostly long term cancer risk in humans, and not so relevant to small animal micro-CT, as we will see. A whole body dose will of course carry more danger of radiation harm than just a dose to the foot. The unit that is used in the field of radiation protection to combine both the local dose, and the bodily extent of irradiation, is the Sievert, or Sv for short. The Sv is the unit of "effective dose".

If this is all too much information, please refer to Appendix 1 at the end of this document that gives further details on calculation of effective dose in Sieverts, including all the tissue weighting factors. With this you can work out what is the dose in Sieverts for any given dose in Grays to any part of the animal. You will see that the Sv dose is always lower – sometimes a lot lower, than the local absorbed dose in Gy, except for whole body scanning where Gy and Sv are the same.

3. How to measure or calculate dose? The on-screen dose-meter and dose calculator

So how do we measure X-ray absorbed dose in the SkyScan 1276 and 1278 scanners? The user is provided with two means of dosimetry, a stand-alone X-ray dose calculator called "CTion" and an on-screen real time dose display. Both use the same method of calculating dose which will be briefly outlined below. Figure 3 shows the menu item and the displayed window for the on-screen dose-meter.

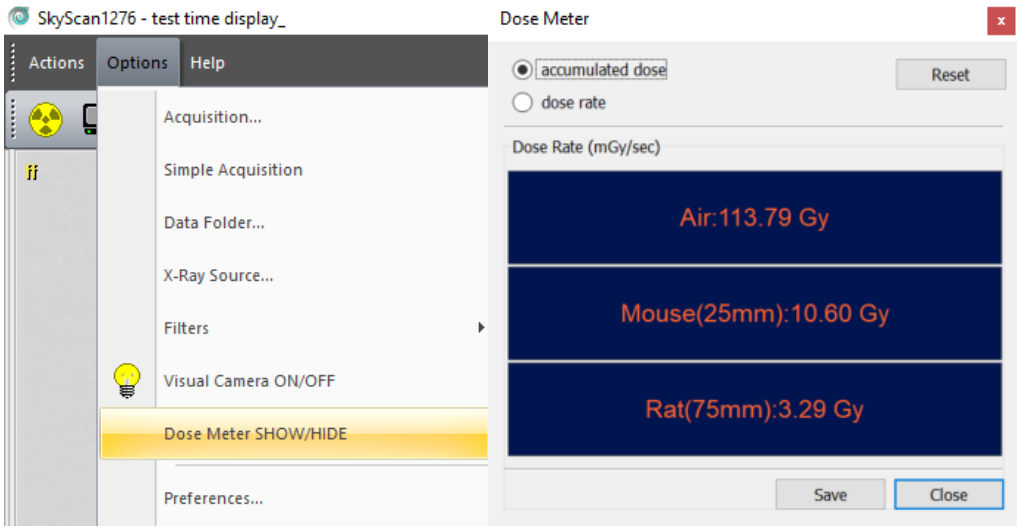


Figure 3. The on-screen dose meter is launched from the Options menu (left) and displays either accumulated dose or dose rate for air, mouse and rat (right).

There are three doses or dose rates displayed, for air, mouse and rat. This is for the important reason that the thickness of the animal part being scanned has a big effect on the absorbed dose. As the thickness increases the dose averaged over all depths gets less, because of attenuation and "self-shielding". The X-rays are depleted with increasing path length and this reduces dose. (From one angle only, dose is highest at the surface and lowest on the opposite side; however over a scan rotation this will average out, so that dose will depend on depth from the surface only.) For the sake of the dose calculations, mice and rats are approximated as tubes of water with 25mm and 75mm diameter respectively. Doses for intermediate

thicknesses of water can be estimated by simple linear interpolation between these given depth values.

The stand-alone dose calculator CTion calculates these same three doses for air, mouse and rat in the same way as in the on-screen dose-meter.

The calculation of X-ray dose in CT*ion* is based on the simulations of X-ray photon energy spectra and dose by the program SpekCalc (http://spekcalc.weebly.com/) created by scientists from the Institute of Cancer Research (London, UK) and McGill University (Montreal, Canada). The details of the emission spectrum and dose-at-distance calculations in SpekCalc have been published[2]. X-rays are assumed to be emitted from an X-ray source using a tungsten (W) target. All SkyScan scanners (and most competitors) use a tungsten target in their X-ray sources.

Absorbed dose rate from X-rays from a laboratory source is determined by these four input parameters in CT*ion*:

- X-ray filter (only if it is in front of the X-ray source, not the camera)
- X-ray source voltage
- X-ray source current
- Distance from the source to the scanned object

All these parameters are reported in the log file of every scan in SkyScan micro-CT systems. So CT*ion* can be used for any scan. This includes past scans and scans planned for the future. (It can also be used to find the dose in systems other than Bruker scanners.) Please note that in some *ex vivo* scanners, filter is in front of the camera, not source. This means that all scans are no filter scans, regardless of filter selected. Systems with filter covering the camera, not source are the SkyScan 1272 and SkyScan 2214. (Note however that for both systems, it is possible to put filters in front of the source instead, improvising with adhesive tape.)

The dose rate is output in units of mGy per minute, so multiplying it by scan time in minutes gives you the total absorbed dose for a scan. Scan duration is also reported in the log file. The intuitive interface for CT*ion* is shown below (figure 4). The program has an accompanying pdf file with full instructions and background information including method of dose calculation.

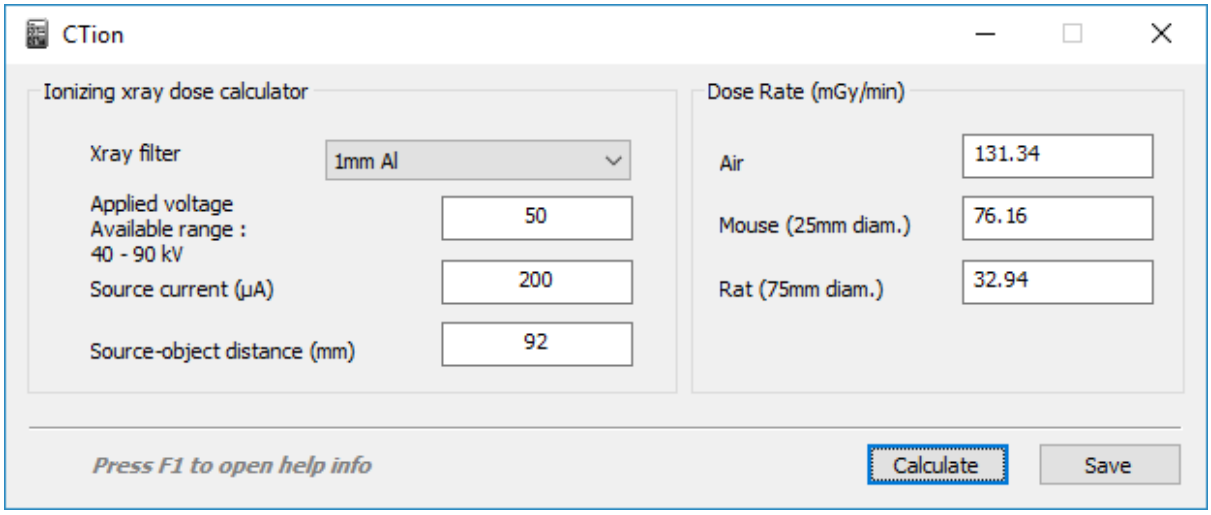


Figure 4. The simple window of the Bruker *CTion* X-ray dose calculator. Four inputs are needed – filter, voltage, current and object-source distance. Doses are calculated for air, mouse and rat.

In the *in vivo* scanners, the dose calculated for a scan is also reported in the log file, for the three depth values (air, mouse, rat).

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58 Dose estimation Air(mGy)=164.15
59 Dose estimation Mouse(mGy)=102.27
60 Dose estimation Rat(mGy)=49.35
61 Study Date and Time=02 Aug 2021  10h:33m:28s
62 Scan duration=0h:1m:19s
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The thickness values that should be used for different *in vivo* scan sites on the mouse and rat are shown with four examples in figure 5. The mouse body in an average adult mouse can be approimated as a 25mm diameter cylinder of water. For both the mouse head (for dental scans for instance) and also the rat hindlimb, a 15mm water diamter can be assumed. The smaller hindlimb of the mouse in the knee region mostly used for bone imaging has a thickness corresponding to about 7.5mm of water.

Figure 5. Different *in vivo* scan scenarios have corresponding tissue thicknesses that should be used for calculating X-ray dose (a) The adult mouse body for thorax or abdominal scan has thickness about 25mm. (b) For mouse hindlimb scans for bone or muscle mass, thickness is about 7.5mm in the knee region. (c) and (d) for both the mouse head and the rat hindlimb the thickness is about 15mm.

4. What is a safe dose for *in vivo* micro-CT scanning?

Let's consider the approximate safe dose for some different scan scenarios. For this we need to consult the published literature on X-ray effects with dose on mice and rats. In general a hierarchy of radiation effects exists on different biological scales, from the molecular-genetic scale to the cell to the tissues and whole organism. At the smallest scale, genetic effects such as expression of many proteins, including heat shock proteins, can be triggered by doses as low as 20-100 mGy [3,4,5]. Such sensitivity might seem to justify extreme caution about radiation exposure. But one must be cautious with such caution! On the scale of the whole cell, cell culture experiments repeatedly find that the X-ray dose needed to slow down or stop division of growing cells is ten times higher, in the range 1.2-1.5 Gy[6,7,8]. And at the higher level of

tissues and the organism, the doses needed to cause measurable tissue damage and eventually death of the animal are another of magnitude higher again at around 10-20 Gy [9,10,11].

The most relevant of these damage thresholds is the one for cells. Doses above one Gy which curtail cell growth and division will affect growing tissues that are studied by micro-CT, such as the hindlimb knee growth plates where chondrocytes divide to produce new bone. This bone growth must not be interfered with by an *in vivo* micro-CT scan.

It should be noted that low doses of ionizing radiation exert positive health effects on mice and other experimental animal models. Studies repeatedly find that doses of a few tens or even hundreds of mGy cause a beneficial stimulation of the immune system resulting in, among other things, suppression of tumour growth and extension of lifespan[12,13]. This phenomenon is well-established and is sometimes called “radiation hormesis”.

For the scan of the mouse or rat hindlimb, the published evidence suggests that bone growth is not changed significantly, over the course of an *in vivo* study, by absorbed doses up to between 500 and 800 mGy. Therefore the upper limit of dose received by the knee should be in this range. So 500 mGy would be a conservative dose limit to apply. The published studies that contribute to this conclusion are shown in table 1, which summarizes the experimental details such the age of the mice or rats, the micro-CT scanner used (mostly SkyScan but some with Scanco) and the number of scans and time interval between them.

Note that one study, Bott *et al.* 2020 (ref. 22) found a negative effect on bone growth at a dose less than 500 mGy – at 460 mGy. However the X-ray dose is probably underestimated in this study. This paper provided all the scan parameters needed for calculation of the X-ray dose using the Bruker X-ray dose calculator, CTion (more on this later). CTion calculated a dose of 1130 mGy for the scans reported by Bott. *et al.* (scan duration 16.383 minutes with protocol of Sacco *et al.* 2017). These authors used a MOSFET personal dosimeter, and these dose meters have an X-ray energy detection window whose sensitivity declines below 30 keV (we know this because we have the same type of detector in Kontich). Bott *et al.* scanned with 40kV applied and 1mm aluminium filter, giving a mean X-ray photon energy of 24.8 keV

– so most of the X-rays from their scans were below 30keV in energy, and the dose therefore under-stated. Their actual dose was well above 500 mGy.

Therefore, an upper safe limit of 500 mGy for bone micro-CT scans seems to be a reasonable conservative interpretation of the available literature, which is quite coherent. For soft tissue, there is not so much literature available on the effect of X-ray dose from micro-CT scans on scanned tissue, as there is for bone. Some examples of studies of soft tissue scan radiation effects are shown in table 2.

There are several published studies of brain effects of X-ray radiation. Silasi *et al.* 2004[14] gave mice that were 45 days old 0.5 Gy of X-rays either by single acute exposure or by 50 mGy daily exposures (over 25 seconds) for 10 days. This was to see the difference between acute and chronic irradiation. Effects of chronic daily irradiation proved to be much more than of acute irradiation with same total dose. Protein expression in the brain was affected in males but not females, with decreased signaling also observed in the male hippocampus. Estrogen appeared to be radio-protective, as ovariectomy in female mice made them susceptible to the same X-ray effects as males. The finding that radiation was more harmful when given as multiple smaller doses than a large single dose is important, indicating that biological hazard from radiation could be increased by several repeated scans compared to a single scan, especially if scans are closely spaced by just one or a few days. A 50 mGy scan would generally be considered very low dose. But when repeated daily for 10 days, the effect clearly becomes additive.

In another study of brain effects of X-rays, Verreet *et al.* (2015)[15] employed a Bruker MRI (Biospec, 9.4T) to perform 3D T2-weighted MRI, diffusion tensor imaging (DTI) on juvenile mice following prior irradiation in utero at 11 days of gestation. Applying X-ray doses of 0 (control), 250, 500 and 1000 mGy, they found that only the highest 1 Gy dose group exhibited hippocampal-dependent spatial learning and memory deficits caused by the radiation, the lower doses being not different from control. This is surprising, that a dose threshold of half a Gray or more should be found for brain effects at 11 days in utero.

The lung is another tissue for which many studies of radiation effects have been done. Foremost among these have been two studies by the lab of Greetje Vande Velde at KUL (the

Catholic University of Leuven, Belgium) employing the SkyScan 1278. In the first[16], mice underwent a single listmode lung scan at 8 weeks age delivering 813 mGy. No lung effects were seen relative to unscanned mice.

The second study from Vande Velde's group [17] gave 8 week old mice a series of 5 weekly scans delivering 500-700 mGy per scan. In this case, while no morphological changes in the lungs were seen, counts of blood lymphocytes and platelets were decreased from the X-ray exposure. The same scan series with X-ray dose reduced to about 200 mGy eliminated even the blood cell changes. Greetje's studies show that a radiosafe threshold can be defined either based on organ level morphological changes (e.g. 700mGy) or alternatively based on cellular or gene expression responses to ionizing radiation (e.g. 200mGy). The latter will always be a lower threshold.

A radiotoxic lung dose was found by Ford *et al.* (2019)[18] who scanned 8-week rats. A scan dose of 470 mGy had no effect but 1.5 Gy caused lung inflammation (assessed by post-mortem histology). This is a useful study indicating a threshold for lung damage near to one Gray. To put these doses from scans in context, Burghardt *et al.* 2020[19] used an experimental SkyScan/Bruker phase contrast *in vivo* scanner to assess lung damage from a very large X-ray dose of 20 Gy to one lung only. (The dose was not from the scanner but other X-ray equipment.) Even after this high dose, it took 20-30 weeks for the lung damage to become established, as is shown in figure 6. It is remarkable that a high dose of 20 Gy, even to one lung only, hardly shortens the lifetime of the mouse.

Miyahara *et al.* (2016)[20] scanned 7 week old mice 3 times per week for 4 weeks, with a very low dose of 16 mGy per scan, 194 mGy in total. No effects were found in body weight, organ weights or blood constituents. This shows that at a low enough dose below 20 mGy, even frequent scanning has no detectable radiation caused effect. So this study at least gives us a "low end marker" of a very low X-ray dose that is radiosafe even by blood cell criteria, even when repeated every few days.

Table 1. Published studies assessing safe and unsafe radiation doses in micro-CT *in vivo* scans of the mouse and rat knee. The upper safe dose seems to lie in the range 500-800 mGy.

Published study	Animal, age at start of experiment	Micro-CT scanner	Absorbed dose per scan, mGy	Scan number and interval	Method of control for radiation effects on bone	Relative radiation effect detected?
Klinck *et al.* 2008[21]	Rats 8 months	Scanco viva40	502.5	6, 2 weeks	Per animal scanned/non-scanned limb comparison	No
Klinck *et al.* 2008[20]	Mice 12 weeks	Scanco viva40	712.4	5, 1 week	Per animal scanned/non-scanned limb comparison	Yes
Laperre *et al.* 2011[22]	Mice 4 or 16 weeks	SkyScan 1076	166-434	3, 2 weeks	Anesthesia only control animals	No
Laperre *et al.* 2011[21]	Mice 10 weeks	SkyScan 1076	776	3, 2 weeks	Anesthesia only control animals	Yes
Longo *et al.* 2016[23]	Rats 13 weeks	SkyScan 1176	603	4, 1 month	Per animal scanned/non-scanned limb comparison	No
Sacco *et al.* 2017[23]	Mice 2 months	SkyScan 1176	222-261[a]	3, 2 months (2,4,6 months)	Per animal scanned/non-scanned limb comparison	No
Sacco *et al.* 2017[24]	Mice 2 months	SkyScan 1176	460[b]	3, 2 months (2,4,6 months)	Per animal scanned/non-scanned limb comparison	No
Bott *et al.* 2020[25]	Mice 2 months	SkyScan 1176	460[b]	4, 1 month (2,3,4,5 months)	Per animal scanned/non-scanned limb comparison	Yes
Mustafy *et al.* 2020[26]	Rats, 4 weeks	SkyScan 1176	830	9 weekly scans from 4-12 weeks age	Per animal scanned/non-scanned limb comparison	No
Mustafy *et al.* 2020[23]	Rats, 4 weeks	SkyScan 1176	1650	9 weekly scans from 4-12 weeks age	Per animal scanned/non-scanned limb comparison	Yes

[a] According to CTion, 272-340 mGy

[b] According to CTion, 713 mGy

Table 2. Published studies assessing safe and unsafe radiation doses in micro-CT *in vivo* scans of the mouse soft tissues and whole body. The upper safe dose seems to be about 500 mGy for adult animals. Protraction or repetition of radiation dose increases its harmful effect relative to the dose amount.

Published study	Animal, age at start of experiment	Micro-CT scanner	Absorbed dose per scan, mGy	Scan number and interval	Method of control for radiation effects	Relative radiation effect detected?
Van de Velde *et al.* 2015[17]	Mice, 8 weeks	SkyScan 1278	813	1 scan (listmode)	Scanned versus unscanned mouse comparison of lung	No
Van de Velde *et al.* 2019[17]	Mice, 8 weeks	SkyScan 1278	540-699 mGy	5 weekly scans	Scanned versus unscanned mouse comparison of lung	Lungs OK, blood cells reduced)
Van de Velde *et al.* 2019[17]	Mice, 8 weeks	SkyScan 1278	180-233 mGy	5 weekly scans	Scanned versus unscanned mouse comparison of lung	Lungs OK, blood cells normal)
Ford *et al.* 2019[18]	Rats 8 weeks	Dual energy, CT Ehrlangen	470 mGy	Single scan	Scanned versus unscanned mouse comparison of lung	No
Ford *et al.* 2019[18]	Rats 8 weeks	Dual energy, CT Ehrlangen	1500 mGy	Single scan	Scanned versus unscanned mouse comparison of lung	Yes (lung inflamm.)
Burghardt *et al.* 2020[16]	Mice 12 weeks	Test X-ray irradiation	20 Gy of single lung	One test irradiation	Irradiated versus non-irradiated lung. Phase contrast CT	Yes
Silasi *et al.* 2004[14]	Mice 45 days	Test X-ray irradiation	500 mGy	500 mGy single or 50 mGy daily 10 days	Irradiated versus non-irradiated. Brain protein expression change, hippocampus signaling decrease	Yes More in males than females; chronic more than single
Verreet *et al.* 2015[15]	Mouse embryo 11 days	Test X-ray irradiation, not micro-CT	500 mGy	One test irradiation	Irradiated versus not, analysis of behaviour and brain function	No
Verreet *et al.* 2015[15]	Mouse embryo 11 days	Test X-ray irradiation, not micro-CT	1000 mGy	One test irradiation	Irradiated versus not, analysis of behaviour and brain function	Yes
Miyahara *et al.* 2016[26]	Mice 7 weeks	Rigaku micro-CT	16 mGy (194 mGy total)	12, 3/week 4 weeks	Irradiated versus not, analysis of body and organ weights and blood	No

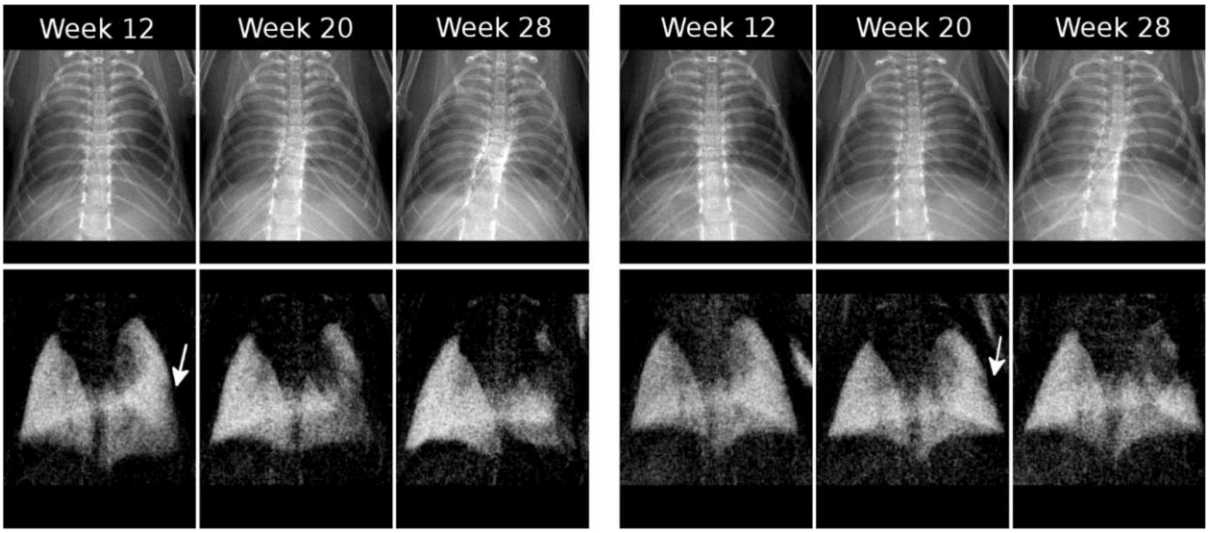

Figure 6. A SkyScan experimental *in vivo* phase contrast scanner obtained these images showing developing damage to one lung (the right as we look at it) from irradiation of that lung only with 20 Gy. It takes 20 weeks for significant damage to be visible. The phase contrast projections give better lung structural information than conventional absorption projections which are obscured by bone. (From Burghardt *et al.* 2020[19].)

In summary, the data discussed here are helpful pointers but not sufficient for precise thresholds of radiation effect. However we can make general conclusions and recommendations for users of *in vivo* micro-CT scanners. These recommendations would be the following:

- For micro-CT scans *in vivo* of the hindlimb, thorax and head as single locations, the dose should not be higher than 500 mGy
- For micro-CT scans *in vivo* of the abdominal region and whole body scans, the dose should not be higher than 250 mGy
- For micro-CT scans *in vivo* of mouse fetuses in pregnant mothers, a conservative dose limit would be 125 mGy, although the published data aren't clear on this
- X-ray doses of 100 mGy or less can be expected to have positive health effects based on immune stimulation including suppression of spontaneous tumours and increased longevity of mice. If a tumour model is being scanned, a tumour-suppressing "radiotherapy" effect is possible from microCT scanning.

Appendix I: Effective dose equivalent, the Sievert

What is a Sievert and how is it different from a Gray?

We need to know what Gray and Sievert mean because micro-CT manufacturers and users sometimes quote X-ray doses in both units. First, the Sievert has the same units as Gray, joules per kilogram. However this effective dose value is modified by (dimensionless) numbers or indices which are selected to give the dose in Sv a weighting that reflects its biological damage potential – known in the trade as "biological effectiveness". There are two such indices. One is the Quality factor Q, that indicates the different biological effectiveness of different radiation types. Thus for alpha particles Q=20, for neutrons Q=6 and for beta particles, gamma rays and X-rays Q=1. In our case, since we are dealing with X-ray only, we don't need to take account of the radiation type quality factor.

The second index is more important, the tissue weighting factor or H_T. This is an adjustment for the different radiobiological sensitivities of different tissues. For example the gonads and parts of the gut have a high radiosensitivity and thus higher H_T, while for skin and bone surface H_T is low. All the body tissues have an H_T weighting value. So to calculate effective dose in Sv, you need to first quantify for each tissue what fraction of this tissue is irradiated in a scan; for instance in a head scan, the tissue fraction of brain irradiated is 100% but the fraction of gonads irradiated would be zero. For each tissue fraction that is irradiated, that fraction is multiplied by the tissue weighting factor H_T. The sum of all these weighted fractions is multiplied by the absorbed dose in Grays, to obtain the effective dose in Sv. The effective dose in Sv to part of the animal can be thought of as the equivalent dose to the whole animal that would cause the same radiation harm.

With this you can calculate Sv from Gy for any scans. The point about the Sv effective dose is that it can be very small and this can be used in a misleading way to claim low scan dose.

Effective dose in Sv is usually less than the absorbed "physical" dose in Gy. Only if the whole body is irradiated, then no calculation is needed and the effective dose in Sv equals the absorbed dose in Gy. For irradiation of less than the whole body, the Sv dose is less than the Gy dose. For a scan such as of a mouse hindlimb, the effective dose in Sv can be very small

indeed. The appendix table 3 shows that when the mouse knee is scanned, the effective dose fraction from the small percentage of the animal's muscle, skin and bone in the knee is only 0.0044. That means that if the knee is scanned – a frequently done scan in bone research – and the knee received a dose of one Gy, the effective dose for this scan would be 4.4 milliSv. Now 4.4 mSv sounds a much smaller dose than 1 Gy, and some micro-CT vendors or even scientists take advantage of this fact by quoting dose in Sv instead of Gy.

However the sievert is not so relevant to micro-CT X-ray dosimetry. It was developed for use in determining cancer risk to humans over a whole lifetime, from small doses of radiation. In micro-CT where the animals are euthanized at the end of a few weeks long study, long term cancer risk is not important. What matters is the local concentration of ionization from X-rays which risks damaging tissues, especially growing cells and tissues that are more vulnerable to ionizing radiation than non-growing. So in short – for micro-CT the local dose in Gy is relevant and the equivalent "effective" dose normalized over the whole body, in Sv, is much less so. The discussion about radiation dose and it's biological risk in micro-CT scanning is about Grays, not Sieverts.

Calculation of the Sievert effective dose

In the absence of detailed dosimetry factors for rodents, we can obtain estimates of effective dose equivalents to rodents by using human tissue weighting factors published by the International Commission on Radiological Protection (ICRP).

We have to take into account the varying biological effects of radiation on a particular tissue (or body part) type, *T*. The same radiation exposure to different parts of the body can have very different results. That is, if the entire body were irradiated with a uniform beam of a single type of radiation, some parts of the body would react more sensitively than others. To take this effect into account, the ICRP has published list of *tissue weighting factors*, denoted $\boldsymbol{W_T}$, for a number of organs and tissues that most significantly contribute to overall biological damage to the body (ICRP *Publication 60,* 1990). Table 3 below gives the values of the tissue weighting factor W_T from ICRP 60.

The ICRP define the integrated effective human-equivalent dose, or *"effective dose equivalent"* denoted $\boldsymbol{H_E}$, for the determination of the whole-body biological damage due to various forms of radiation exposure in different parts of the body. This effective dose equivalent is given as follows:

$$H_E = \sum_T W_T \times D_T \times Q \qquad (1)$$

where W_T is the ICRP's tissue weighting factor for the type of tissue or body part T, and D_T is the dose in Gy for tissue T and Q is the quality factor for the type of ionizing radiation. The units of H_E are Sieverts, *Sv*. Note that Grays and Sieverts have the same physical units of absorbed energy per mass, joules/kg. Essentially the effective dose equivalent indicates the radiation probabilistic harm caused by irradiation of a restricted part of the body by ionizing photons or particles, expressed as the corresponding dose of low LET radiation given uniformly to the whole body that would cause the same degree of radiation probabilistic harm. Note that the quality factor Q for all X-rays and gamma rays is 1, while for neutrons it is 6 and alpha particles 20. Thus for X-ray dosimetry and dose equivalent calculations Q does not play a role.

Tissue weighted effective dose equivalents are shown in table 3 for four typical rodent micro-CT scan scenarios, head, upper body, lower body and knee. Tissue fractions have been calculated by reference to ICRP publication 70 (1995), Radiation protection basic anatomical and physiological data.

As an example for interpreting these data, a dose in mGy received by the knee should be multiplied by the weighting factor of 0.0044 to calculate an effective dose equivalent in mSv – that is, the dose (low LET) delivered uniformly to the whole body of the rodent, that would cause the same radiation harm as the dose delivered to the rodent knee only.

Table 3. Tissue weighted dosimetry of the rodent

Tissue	ICRP Tissue weighting factor H_T	Fraction of tissue, head scan	H_T head	Fraction of tissue, upper body scan	H_T upper body	Fraction of tissue, lower body scan	H_T lower body	Fraction of tissue, knee	H_T knee
Gonads	0.2	0	0	0	0	1	0.2	0	0
Bone Marrow	0.12	0.085	0.0102	0.375	0.045	0.48	0.0576	0.02	0.0024
Colon	0.12	0	0	0	0	1	0.12	0	0
Lung	0.12	0	0	1	0.12	0	0	0	0
Stomach	0.12	0	0	0	0	1	0.12	0	0
Bladder	0.05	0	0	0	0	1	0.05	0	0
Breast	0.05	0	0	0.3	0.015	0.7	0.035	0	0
Liver	0.05	0	0	0	0	1	0.05	0	0
Esophagus	0.05	0	0	1	0.05	0	0	0	0
Thyroid	0.05	1	0.05	0	0	0	0	0	0
Skin	0.01	0.2	0.002	0.3	0.003	0.3	0.003	0.05	0.0005
Bone Surface	0.01	0.14	0.0014	0.3	0.003	0.25	0.0025	0.025	0.00025
Remainder[(a)]	0.05	0.1	0.005	0.4	0.02	0.4	0.02	0.025	0.00125
Total:	1		0.0686		0.256		0.6581		0.0044

a. Remainder: Adrenals, brain, upper large intestine, small intestine, kidney, muscle, pancreas, spleen, thymus, and uterus.

Appendix I: References

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5. Appendix 2: Validation of the CTion dose calculator by comparison with published dose measurements

How do we know that the X-ray doses calculated by CTion are correct? To test this we have compared the results of CTion with dose measurements by a number of groups. In published research journal articles there is sometimes sufficient information given to allow dose measured by the authors to be compared by a dose calculated by CTion. (For instance if the scanner used is a SkyScan model, then we know the source-object distance even if it's not reported.)

The results of this intercomparison are given below both in table 4 and figure 7. Doses calculated retrospectively for published studies (and one unpublished set of measurements) by CTion were sometimes higher and sometimes lower than the published instrumentally measured doses. However figure 7 shows that the average linear regression of the CTion measurements against the published values (the blue dotted line) lies very close to the line representing equality of the measured and the CTion-calculated values (the red line). Overall these intercomparison data represent a validation of the accuracy and consistency with published measurements of the CTion X-ray dose calculator.

Table 4. Some publications using *in vivo* microCT state the four settings needed for dose calculation: filter, voltage, current and scan duration. These allow direct comparison with the dose calculated by CTion, as shown.

Publication	Part of animal scanned	In-vivo microCT dose	Mean X-ray photon energy keV	Method of dose measurement / calculation	CT-ion calculated dose (tissue diameter)
Berghen *et al.* 2019 (radiosafe…)[17]	Mouse thorax	540	28.2	Ionisation chamber	666 (25mm)
Berghen *et al.* 2019 (radiosafe…)[17]	Mouse thorax	699	28.2	TLD in phantom	666 (25mm)
Berghen *et al.* 2019 (radiosafe…)[17]	Mouse thorax	585	28.2	RL probe in phantom	666 (25mm)
Mustafy *et al.* 2018 (Can repeated…)[25]	Rat tibia	830	32.6	UNFORS PS-2 patient skin dosimeter	990 (15mm)
Mustafy *et al.* 2018 (Can repeated…)[25]	Rat tibia	1650	32.6	UNFORS PS-2 patient skin dosimeter	1820 (15mm)
Mustafy *et al.* 2018 (Can repeated…)[25]	Rat tibia	2470	32.6	UNFORS PS-2 patient skin dosimeter	2810 (15mm)
Sacco *et al.* 2017 (Repeated irradiation …)[23]	Rat tibia	222	28.1	MOSFET skin dosimeter	351 (15mm)
Sacco *et al.* 2017 (Repeated irradiation …)[23]	Rat tibia	460	24.8	MOSFET skin dosimeter	950 (15mm)
Vande Velde *et al.* 2015 (Longitudinal *in vivo* … radiotoxicity)[15]	Mouse thorax	813	25.6	TLD calibrated by ionisation chamber, placed inside dead mouse thorax	678 (25mm)
Laperre *et al.* 2011 (Development of u-CT protocols…)[21]	Mouse hindlimb	434	28.1	Ionisation chamber	373 (7.5mm)
Laperre *et al.* 2011 (Development of u-CT protocols…)[21]	Mouse hindlimb	776	25.6	Ionisation chamber	1303 (7.5mm)
Botter *et al.* 2011 (Osteoarthritis induction leads…)[27]	Mouse himdlimb	880	24.8	Ionisation chamber (for air)	675 (0mm)
Willikens *et al.* 2010 (Longitudinal *in vivo* …)[28]	Mouse body	386	25.6	TLDs placed in mouse organs	304 (25mm)
Cao *et al.* 2008 (respiratory gated microCT…)[29]	Mouse body (air)	111	24.8	TLD	150 (air)
Bruker Ettlingen Si78 (unpubished)[30]	(Air)	2286	24.8	Nomex detector (ionisation chamber)	1552 (air)
Bruker Ettlingen Si78 (unpubished)[30]	(Air)	1962	28.2	Nomex detector (ionisation chamber)	1384 (air)
Bruker Ettlingen Si78 (unpubished)[30]	(Air)	1116	32.7	Nomex detector (ionisation chamber)	930 (air)

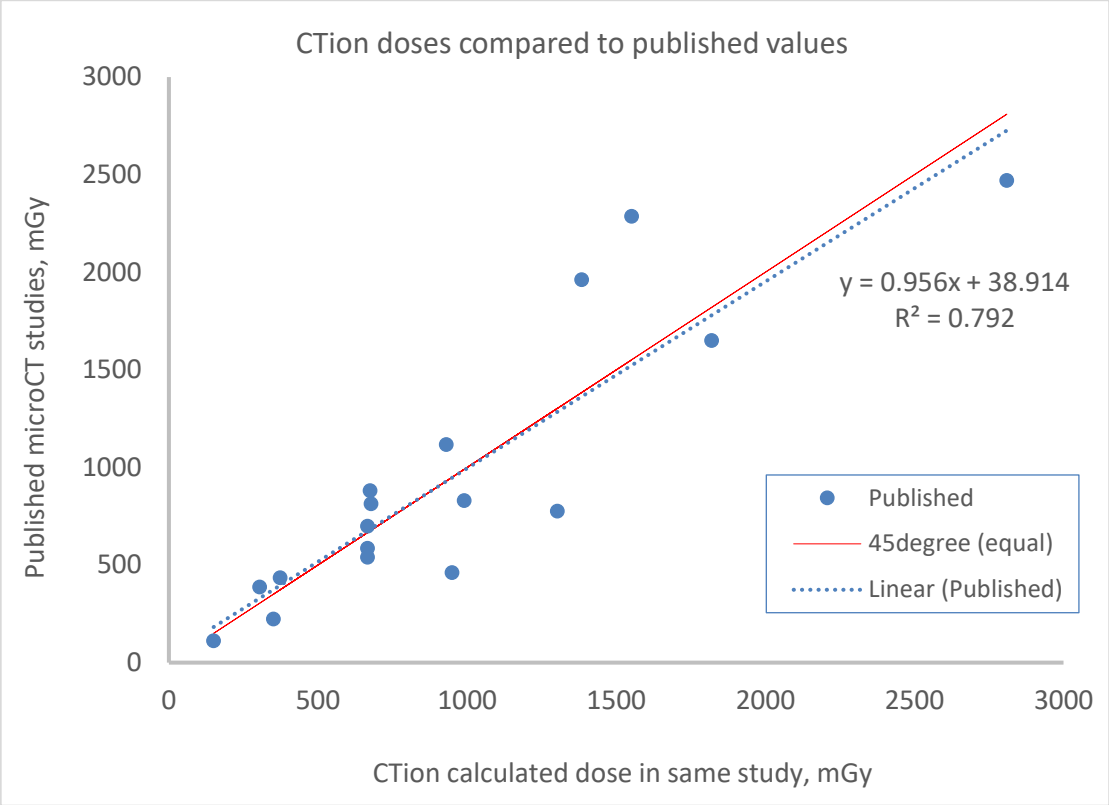

Figure 7. An XY plot of the data in table 4, showing published dose measurements for microCT scanners in the y axis (vertical) and corresponding dose calculation by CTion in the x axis (horizontal). The dotted blue line representing the linear regression between the published dose measurements and CTion calculations, lies close to the red line representing equality between the two.

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