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The potential influence of circadian rhythm on the biodistribution of ^{131}I

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Thesis:	30 hp
Program:	Medical Physicist Programme
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Supervisors:	Eva Forssell-Aronsson, Britta Langen and Johan Spetz
Examiner:	Magnus Båth
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Abstract

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Purpose: The purpose of this study was to investigate the potential influence of circadian rhythm on the biodistribution and biokinetics of ^{131}I in mice.

Theory: ^{131}I is a well-established isotope in radiotherapy. The thyroid is both a target organ when treating thyroid cancer with unbound ^{131}I, but also a risk organ in in ^{131}I-labelled radionuclide therapy. It is therefore important to have a good knowledge of the biodistribution in order to correctly estimate the absorbed dose to the organ. Many biological functions in living organisms follow the circadian rhythm. Nevertheless, the influence of circadian rhythm is generally neglected in biodistrinution studies of radionuclides. To the best of our knowledge, no study has investigated if the biodistribution and biokinetics of ^{131}I exhibit dependency on circadian rhythm.

Method: The concentration of radioactivity in various organs and tissues was studied at different time points after i.v. injection of ^{131}I. The effect of circadian rhythm was studied by varying the time of day of administration. Male C57BL/6N mice were i.v. injected with 140-200 kBq ^{131}I at 12 am or 4 pm and killed after 1 h, 4 h, 8 h, 18 h, 24 h, 3 days and 7 days after injection.

Result: Statistically significant difference in activity concentration between injection performed at 12 am and 4 pm was observed for at least one time point after injection for all investigated organs and tissues. Highest activity concentration was found in the thyroid and maximum was reached at different time points after injection, i.e. 2700 %IA/g at 18 h for injection performed at 12 am and 4500 %IA/g at 4 h for injection performed at 4 pm. Statistically significant difference was observed at 18 h after injection. The result demonstrated that the biodistribution of ^{131}I in mice may be influenced by the time of day when the radionuclide is administered.

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Introduction

In nuclear medicine, radionuclides are used to diagnose and treat many different types of cancer (Kramer-Marek & Capala, 2012; Kohlfürst, 2012; Greene & Wilkinson, 2015). The basic principle is to administer targeting radionuclides or radiopharmaceuticals to the patient. Tumor cells have receptors, transporters, or antigens that can bind these radiopharmaceuticals and usually internalize them into the cell where the radionuclide is then accumulated. The tumor expresses these receptors, transporters or antigens at a much higher level than normal tissue; consequently, the uptake in tumor tissue is much higher than in normal tissue. In contrast to external beam therapy, nuclear medicine makes it possible to treat metastasized cancer disease. Using a radionuclide that emits gamma or positron radiation also makes it possible to detect where the tumors are located. In therapy, the aim is to create irreparable damage in malignant tissue by delivering high absorbed dose. It is also important to limit the radiation to the target tissue to be able to minimize the exposure of normal tissue. To best meet these requirements, the administered radionuclide has to have suitable physical properties such as a sufficiently high linear energy transfer (LET) to produce DNA damage. The particle range must be suitable for the mean average tumor size and the gamma component of the decay has to be low (Uusijärvi *et al*., 2006; Bernhardt *et al*., 2001).

Iodine is a well-established element used in this area of medicine. There are 37 known isotopes of iodine and only one of them, i.e. ^{127}I, is stable (The Lund/LBNL Nuclear Data Search, 1999). ^{131}I is a commonly used isotope in therapy due to its favorable decay properties. The radionuclide disintegrates to ^{131}Xe by β-decay with a mean energy of 190 keV per decay (MIRD, 2006). Due to the short maximum range of respective β-particles with a continuous slowing down approximation (CSDA) range of about 4 mm in water (ICRU, 1984), most of the energy will be absorbed locally and the dose to the surrounding tissue is minimized. ^{131}I has a gamma component with the energy of 364.5 keV (83%) (LNHB, 2014). This allows for detection using a gamma camera or gamma counter. Furthermore, the half-life of ^{131}I is 8.03 days which is an appropriate time for clinical use (MIRD, 2006).

In radionuclide therapy, ^{131}I is administered in free form as iodide or bound to a carrier molecule depending on the desired target tissue. ^{131}I has similar biochemical properties as the stable isotope and will therefore be distributed in the body in a similar way.^{131}I will accumulate in the thyroid if it is administered in unbound form or if it is liberated from its carrier molecule. This results in irradiation of the thyroid and consequently cell death in the tissue. The thyroid is therefore considered a major risk organ in ^{131}I-based targeted radionuclide therapy. This property is utilized when irradiation of thyroid-tissue is desired. One example is hyperthyroidism, which is treated by administration of unbound ^{131}I (Lee, 2012). Patients with differentiated thyroid cancer where the tumor expresses enough amounts of sodium-iodide symporter NIS can also be treated in a similar way. An example of ^{131}I-labeled radiopharmaceutical is metaiodo-benzylguanidine (^{131}I-MIBG), which is an analog of norepinephrine and therefore used in treatment of e.g. pheochromocytoma, paraganglioma and neuroblastoma (Kayano & Kinuya, 2015; Forssell-Aronsson *et al*., 2006). Exposure to ^{131}I can also occur from the environment at nuclear accidents. The Chernobyl accident released approximately $1.8 \cdot 10^{18}$ Bq of ^{131}I and several years after the accident an increase of thyroid cancer in children living in Belarus was reported (McLaughlin *et al*., 2012; Kazakov *et al*., 1992). Another recent example is the Fukushima accident which had an estimated release of ^{131}I in the order of 10^{17} Bq (McLaughlin *et al*., 2012).

The thyroid (*glandula thyroidea*) is an endocrine gland that uses iodine to produce hormones. It consists of two lobes localized along both sides of the trachea, just below the larynx. It is one of the largest endocrine organs in the body and weighs roughly 19 grams in adults (Bisi *et al*., 1989). Thyroid tissue consists of large spherical structures called follicles, as well as blood vessels and connective tissue. The two thyroid-specific cell types are follicular cells and C-cells. The follicular cells surround the follicular lumen, which is filled with protein-containing fluid called the colloid. In the connective tissue around the follicles are the C-cells with the primary function to secrete calcitonin. The main function of the thyroid is to produce and secrete the hormones thyroxine (T4) and triiodothyronine (T3). These hormones regulate many physiological processes in the body, such as stimulation of long bone growth and regulation of body heat. Most commonly, thyroid hormones, T3 and T4, are associated with metabolism as they are essential for regulation of normal tissue metabolism (Yen, 2001). The production of the T3 and T4 hormones takes place inside the follicles. Iodide ions (I^-) are transported from the blood into the follicular cells via NIS. Inside the lumen, one or two iodide ions are bound to the amino acid tyrosine, creating T3 or T4 (Yen, 2001). The hormones are then stored in the colloid within the lumen. The production of T3 and T4 is regulated by a system with both negative and positive feedback loops (Yen, 2001). The hormones are secreted via endocytosis into the follicular cells and further diffuse through the cell membrane into the blood (Haug *et al*., 2007). The synthesis and secretion are stimulated by the thyroid-stimulating hormone (TSH), which is produced and secreted by the anterior pituitary gland in the brain (Yen, 2001). Defects in the thyroid can lead to elevated or reduced levels of thyroid hormones resulting in disorders called hyper- and hypothyroidism, respectively (Yen, 2001).

In order to determine potential risks in both medical and hazard exposure to radioactive iodine, a known dose-response in normal tissue is necessary. It is therefore important to have accurate knowledge of the biodistribution of the radionuclide. Biokinetics are often studied using animal models and the biodistribution of ^{131}I has been studied in many animal species, such as mice (Garg *et al*., 1990), rats (Spetz *et al*., 2013), hamsters (Peronace & Houssay, 1970) and rabbits (Oddie *et al*., 1965).

Many biological functions in living organisms are regulated in an oscillating manner. The body is subject to many different cycles of varying length, such as seasonal changes and cycles with a periodicity of approximately 24 h, the latter of which is called circadian rhythm. Heart rate, sleep/wake cycle, body temperature, as well as secretion of hormones like TSH, insulin, and melatonin are but few examples of processes that follow a circadian rhythm (Schulz & Steimer, 2009). These time-related processes are regulated by a system of endogenous biological clocks, i.e. a time-keeping structure (Reppert & Weave, 2002). In mammals, the suprachiasmatic nucleus (SCN) is the master clock and major pacemaker (Schulz & Steimer, 2009). The SCN is a part of the hypothalamus and is located in the area above the optic chiasma (Reppert & Weave, 2002). The master clock needs to be reset regularly to be able to indicate time correctly. The clock is reset by exogenous stimuli from the environment called zeitgebers (time-givers). Without zeitgebers, the circadian rhythm would be free to run with a period different from 24 h. Social activity and food intake can serve as zeitgebers, but the main zeitgeber is light. When light reaches the retina, a stimulating signal is sent to the SCN via the retinohypothalamic tract (RHT) (Reppert & Weave, 2002). In absence of the light-signal, the SCN will signal to the pineal gland to secrete nocturnal melatonin. This ensures synchronization of circadian rhythm with the day and night time. In turn, the SCN synchronizes the timing of oscillators in other parts of the brain and also in peripheral organs like the liver, the heart, and the kidneys (Schlibler & Sassone-Corsi). Genes that are responsible for control of circadian rhythm are expressed in several tissues in the body (Reppert & Weave, 2002). These genes are called molecular clock genes and are stimulated by the SCN in many

ways. For example, the *PER1* gene is activated by the SCN via adrenal secretion of glucocorticoids (Reppert & Weave, 2002). The circadian cycle in the cell is generated by proteins that either stimulate or inhibit their own synthesis from the molecular clock genes. For instance, PER and CRY proteins are regulated by negative feedback loops, i.e. they inhibit their own synthesis from the *PER* and *CRY* genes (Reppert & Weave, 2002).

The influence of circadian rhythm is generally neglected when radiation-induced effects are studied, and few studies have been performed to address this important biological parameter. One study published in 1971 demonstrated that the accumulated activity of ^{131}I in the thyroid in mice is influenced by circadian rhythm (Walinder, 1971). However, the study focused only on the thyroid and did not investigate the effect of circadian rhythm on the biodistribution and biokinetics of ^{131}I in other tissues. A recent study on radiation-induced gene regulation in mouse tissues showed that both the intensity and biological function of transcriptomic responses to i.v administered ^{131}I are influenced by which time of the day the radionuclide was administered (Langen *et al*., 2015). To the best of our knowledge, no study has investigated if the biodistribution and biokinetics of radionuclides in general, or of ^{131}I in particular, also exhibit dependency on circadian rhythm.

Aims

The purpose of this work was to investigate if circadian rhythm affects the biodistribution and biokinetics of ^{131}I in mice.

In order to validate accuracy of gamma counter measurements, the aim was also to investigate if self-attenuation in the sample and potential geometric effects needs to be compensated for in data analysis.

Materials and methods

Animal experiments were performed to investigate the potential effect of circadian rhythm on the biodistribution and biokinetics of ^{131}I in mice. The radioactivity in various organs was studied at different time points after radionuclide administration. The effect of circadian rhythm was studied by varying the time of day of ^{131}I administration.

Radionuclide and radiation detectors

^{131}I was obtained from GE Healthcare (Braunschweig, Germany) as $Na^{131}I$.

^{131}I activity in samples was measured using two Wallac 1480 Wizard® 3" NaI (Tl) gamma counters, produced by Wallac Oy, Turku, Finland. Gamma counter with serial number 4800361 will be referred to as gamma counter 1 and gamma counter with serial number 4800173 will be referred to as gamma counter 2. Measurements were performed with a pre-installed protocol for ^{131}I with the energy window of 260–430 keV for both detectors. Decay properties of ^{131}I are shown in Table 1.

A CRC-15 dose calibrator ion chamber (Capintec, IA, USA) with known calibration was used as reference detector to determine the activity concentration in the stock solutions containing activity higher than 1 MBq.

Table 1: Data of β^- and γ transitions for ^{131}I with yield >1%. Values are adapted from LNHB (LNHB, 2014)

Radiations	Yield [%]	Energy [keV]
β-	2.13	248
β-	7.20	334
β-	89.4	606
γ	6.63	80.2
γ	1.09	164
γ	6.45	284
γ	83.1	364
γ	7.15	637
γ	1.79	723

Calibration of the gamma counters

To find the relationship between the measured counts per second (cps) and the activity in the sample, a calibration of the gamma counters was conducted. Radioactive solutions of 1 ml with varying concentration of ^{131}I were prepared by two-fold serial dilution, which generated solutions with linear decreasing activity from 800 kBq to 1.5 kBq. The activity in the stock solution was determined with the ion chamber before dilution. Measurements in the gamma counter were corrected for dead time using the dead time factor achieved from the detector.

Calibration factors for ^{131}I was obtained by a linear fit to the measured cps in the gamma counters vs. the activity in the samples measured with the ion chamber. The calibration factors were used to interpret the measured data in the animal experiments.

Measurement corrections

Self-attenuation of ^{131}I in the sample was investigated by measuring tissue equivalent cylindrical phantoms of varying sizes containing ^{131}I. The phantoms consisted of 2% agarose gel with an added Na^{131}I solution diluted in ultrapure water (Merck Millipore, Darmstadt, Germany). Phantoms were prepared in inverted 1.5 ml cryogenic vials to achieve a simple cylindrical geometry.

Self-attenuation was investigated for constant activity and for constant activity concentration at varying phantom volume. Phantoms containing similar activity (25 kBq ^{131}I) were prepared with nominal volumes of 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 5, 10 and 15 ml. The activity in the phantoms was chosen with regard to the sensitivity in the ionization chamber and expected activity in the organ samples. To determine that the phantoms were of correct volume, the phantoms were weighed. Phantoms with the same activity concentration of 100 kBq/ml and a nominal volume of 0.05, 0.1, 0.15, 0.25, 0.3, 0.5, 0.75 and 1 ml were also made. The phantoms were weighed and mass was used as measure instead of nominal volume. All phantoms were placed in 20 ml scintillation vials and measured in the gamma counter.

To examine whether the geometric location of the sample in the scintillation vial affected the result, measurements were performed with the phantoms of varying volumes positioned at different locations in the measurement apparatus. The phantoms were placed at the edge of the scintillation vial which was measured in both gamma counters at four different orientations (Figure 1).

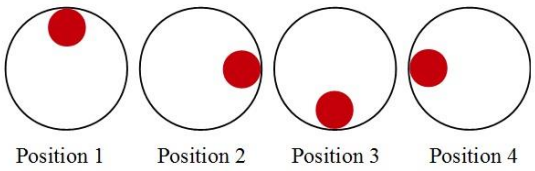

Figure 1: Positions of the phantoms in the investigation of geometric effect. The figure shows the position in the scintillation vial seen from above

Animal experiments

Animals used in this study were nine to ten weeks old male C57BL/6N mice (Charles River Laboratories International, Inc., Salzfeld, Germany) weighing 21.4−25.6 g. The mice were kept under standard laboratory day and night cycle, i.e. dark from 6:00 pm to 6.00 am. Water and laboratory food with iodine concentration of 0.87 µg/g were given *ad libitum*. The study was approved by the Ethics Committee for Animal Research in Gothenburg (no. 146-2015).

Radionuclide administration

Syringes (0.01−0.1 ml with 0.4x20 mm cannulas) containing ^{131}I-solutions (0.1 ml) with a nominal activity of 150 kBq were prepared from a stock solution with an activity concentration that was determined with the CRC-15 dose calibrator ion chamber.

A certain amount of ^{131}I binds to the plastic on the inside surface of a syringe, i.e. the injected volume has a lower activity concentration than the solution that was added to the syringe. A control test of this effect with three syringes showed that an added activity concentration of 1.5 MBq/ml resulted in a concentration of about 1.1 MBq/ml in the injected volume. The control test was repeated with an higher activity concentration of 1.9 MBq/ml, resulted in a concentration of about 1.8 MBq/ml in the injected

volume. To compensate for this effect and assure a chosen activity concentration of 1.5 MBq/ml upon injection, the activity concentration in the stock solution was set to 1.7 MBq.

The potential dead space effect in syringes was investigated by weighing of the entire ejected solution from three control syringes and found to be irrelevant (data not shown).

To determine the actual administered activity of the syringes used for administration, each syringe was weighed before and after injection. Three control syringes were used for each injection series to determine the activity concentration of the injected volume. The injected activity for each group is given in Table 2. Due to errors in weighing of three syringes, a mean weight of injected ^{131}I solution was calculated and used in calculation of administered activity for these syringes. The mean weight was calculated from 22 syringes prepared at the same time. The mice that were injected at 4 pm and killed after 4 h, 8 h, and 3 days were given a somewhat higher activity than nominally determined, i.e. around 200 kBq. Otherwise, the injected activity was close to 150 kBq.

Table 2: Mean values for the injected activity for each group with SEM (italics)

Group	Injected activity [kBq]	
Injection at 12 am		
1 h	160	*3*
4 h	160	*5*
8 h	150	*4*
18 h	145	*6*
24 h	150	*5*
3 days	160	*5*
7 days	160	*7*
Injection at 4 pm		
1 h	140	*4*
4 h	200	*7*
8 h	190	*8*
18 h	170	*2*
24 h	150	*2*
3 days	190	*3*

Biodistribution and biokinetics

The animals were divided into two main groups according to the time of day of ^{131}I administration. The groups were i.v. injected with ^{131}I in the tail vein at 12:00 am or 4:00 pm, respectively. Each main group was divided into seven groups with five mice per group. The animals were killed by cardiac puncture under anesthesia with sodium pentobarbital (APL, Sweden) after 1, 4, 8, 18, 24, 72, or 168 hours following injection. The thyroid, salivary glands, lungs, heart, spleen, liver, kidneys, and stomach were excised and blood and gastric contents were sampled. Several millimeters of the large intestine (starting from the sigmoid colon) and of the small intestine (staring from the duodenum) were excised and contents from the large and small intestine were collected. Sample weight was measured directly after excision. Due to unreasonably high weights of some thyroid samples, an average weight of 3.9 mg (SD=0.5) was calculated from 25 samples with reasonable weight in accordance with literature

(Walinder, 1971). The average weight was used as weight for samples with higher weight than average (plus 1 SD). The activity in the samples was measured with the gamma counters and corrections were done for background and dead time. Measurements were performed with such time that a minimum 1000 counts over background were achieved. All measured activities were decay corrected to time of injection.

The activity concentration in each organ and tissue at different times after injection was calculated as percent of injected activity per organ weight (%IA/g). The ^{131}I activity in the total blood volume was calculated using an estimation of total blood volume with 0.0847 ml per gram body weight (Riches *et al*., 1973) and a density of 1.057 g/ml (Riches *et al*., 1973).

Statistical analyses

Uncertainties in the measurements are given as the standard error of the mean ($SEM_{\bar{x}}$):

$$SEM_{\bar{x}} = \frac{SD}{\sqrt{n}} = \frac{\frac{1}{n-1}\sum_{i=1}^{n}(x_i-\bar{x})^2}{\sqrt{n}},$$

where x_i is the value of sample i, $\bar{x}$ is the mean value of the samples and n is the number of the samples in the group.

Student's t-test was used to determine the statistical significance of differences between the groups with different time of day of ^{131}I administration. Statistical significance was considered for probabilities higher than 95% ($p<0.05$). Student's t-test was also used to determine the statistical significance of differences in the investigation of volume and geometric effects. Probabilities higher or equal to 99% ($p\leq 0.01$) were considered statistically significant.

Results

Calibration of the gamma counters

The result from the calibration of the gamma counters is shown in Figure 2. The two detectors showed a different relationship between the measured cps and activity in the sample.

The calibration curve for gamma counter 1 (Figure 2a) is a mean value of three calibrations with activities from 1.5 kBq up to 800 kBq. The relationship can be considered to be linear up to about 11 000 cps which corresponds to 25 kBq. For higher activities, the achieved dead time factor failed to compensate for the loss of counts, resulting in a nonlinear response. The dead time factors at 25 kBq were 11%, 11% and 12% and, due to the linearity, a dead time factor up to 12 % was considered acceptable for further measurements in this detector. The calibration factor ($k = 444.09$ cps/kBq) was obtained from linear approximation to the data up to 25 kBq.

For gamma counter 2 (Figure 2b), the response can be considered to be linear up to about 14 000 cps which corresponds to 25 kBq. The graph is a mean value of two calibrations with activities from 1.5 kBq up to 800 kBq. The dead time factors at 25 kBq were 15% and 12% therefore a dead time factor up to 15 % was accepted for measurements in this detector. The calibration factor ($k = 521.07$ cps/kBq) was obtained from linear approximation to the data up to 25 kBq.

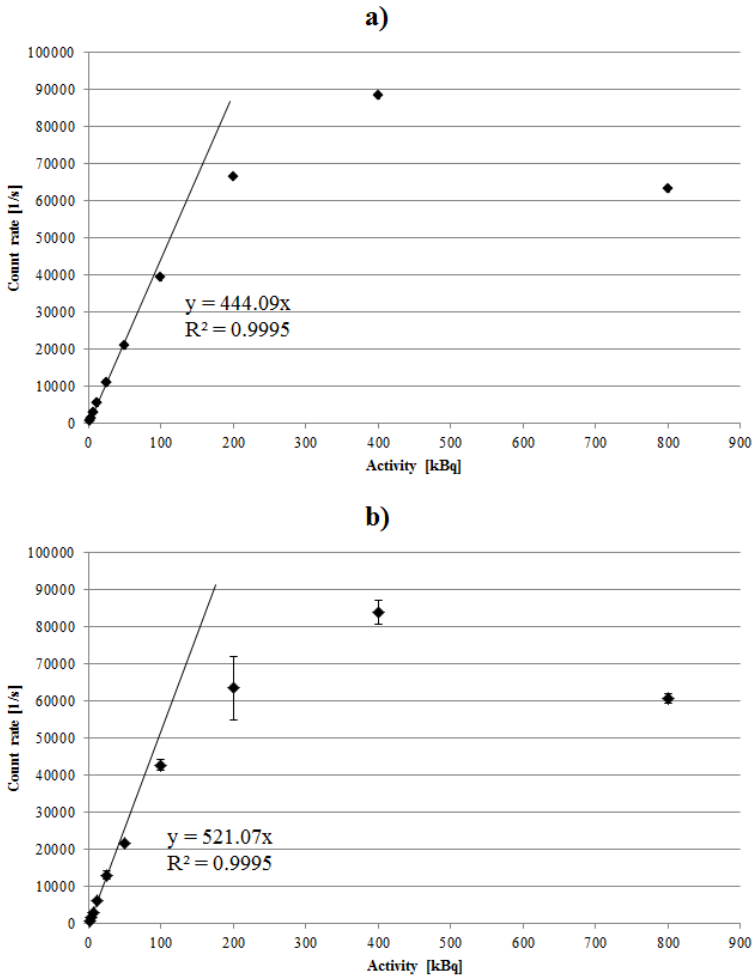

Figure 2: Calibration of gamma counters. a) Calibration of gamma counter 1, data points are mean values from three dilution series. **b)** Calibration of gamma counter 2, data points are mean values calculated from two dilution series**.** The graphs show the relationship between cps measured in the gamma counters and ^{131}I activity measured with the ionization chamber as reference detector. Linear decreasing concentrations of ^{131}I were prepared by means of a two-fold serial dilution with a final volume of 1 ml. Error bars indicate SEM; the black line is a linear curve fit to the data points up to 25 kBq

Measurement corrections

Volume effect

The result from the investigation of self-attenuation of ^{131}I in phantoms with varying size and a similar ^{131}I activity concentration of 100 kBq/ml is shown in Figure 3. The relationship between the measured cps and mass of the phantoms is linear and accordingly, no self-attenuation was observed. Measurements were performed with gamma counter 2.

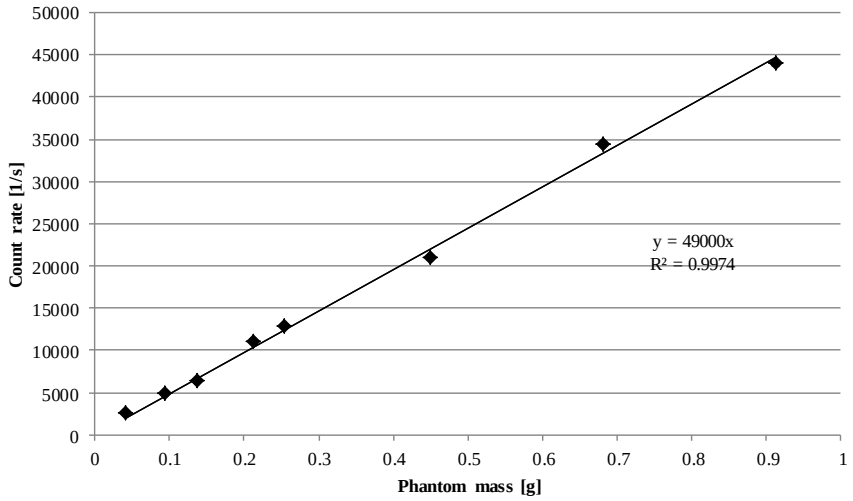

Figure 3: Relation between phantom volume and cps at similar ^{131}I activity concentration. Tissue equivalent phantoms consisted of conical volumes of 2% agarose gel containing 100 kBq/ml ^{131}I. The black line is a linear approximation to the measured values. Measurements were performed with gamma counter 2

The result from the investigation of self-attenuation of ^{131}I in phantoms with varying size and a similar ^{131}I activity of 25 kBq is shown in Figure 4. Phantoms were prepared in several series at different nominal volumes; to assure sufficient accuracy of phantom preparation, phantom mass was measured as presented in Table 3.

Table 3: Weight and volume of the phantoms consisting of 2% agarose gel containing 25 kBq ^{131}I. SEM (italics) are given for the calculated mean values

Volume [ml]	Phantom weight [g]						Mean weight [g]
0.05	0.03	0.026	0.032	0.047	0.036	0.029	0.033 *0.003*
0.1	0.077	0.089	0.085	0.084	0.081		0.083 *0.002*
0.25	0.20	0.19	0.21				0.20 *0.01*
0.5	0.45	0.43	0.45				0.44 *0.01*
0.75	0.86	0.75	0.69				0.77 *0.05*
1	0.89	0.92	0.94				0.92 *0.02*
2	1.9	1.9					1.9 *0.0*
5	4.8						4.8
10	9.5	9.5					9.5 *0.0*
15	15	14					15 *1*

The determined weight was overall lower than the nominal volume.

Figure 4a shows a compilation of the measured cps for all series and the mean values are shown in Figure 4b. Large deviations in the measured cps were observed for the 0.05 and 0.1 ml phantoms. Statistical analysis showed no significant differences in the measured cps for the different volumes.

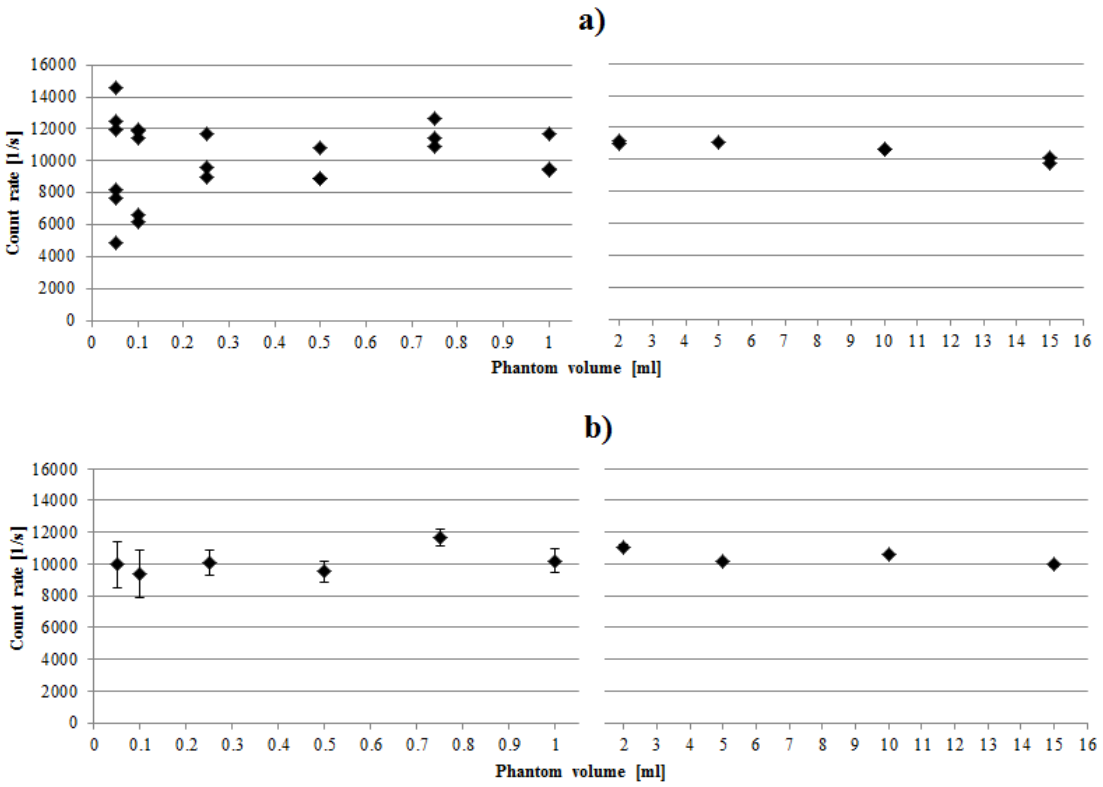

Figure 4: Relation between measured signal (cps) and phantom volume at constant ^{131}I activity. a) Tissue equivalent phantoms consisted of conical volumes of 2% agarose gel containing 25 kBq ^{131}I. **b)** Mean values of the phantoms in a). Error bars indicate SEM. Measurements were performed with gamma counter 2. Note the change of scale on the x-axes

Geometric effect

The result from the investigation of a potential geometric effect in the measurement apparatus is shown in Figure 5. The presented data are mean values of the phantoms with an activity of 25 kBq ^{131}I positioned according to Figure 1. The measurements were performed in both gamma counters. The results show no statistically significant difference between the four positions, and consequently no geometric effect was observed. Therefore, no correction for geometric effect was, therefore, made in the animal experiments

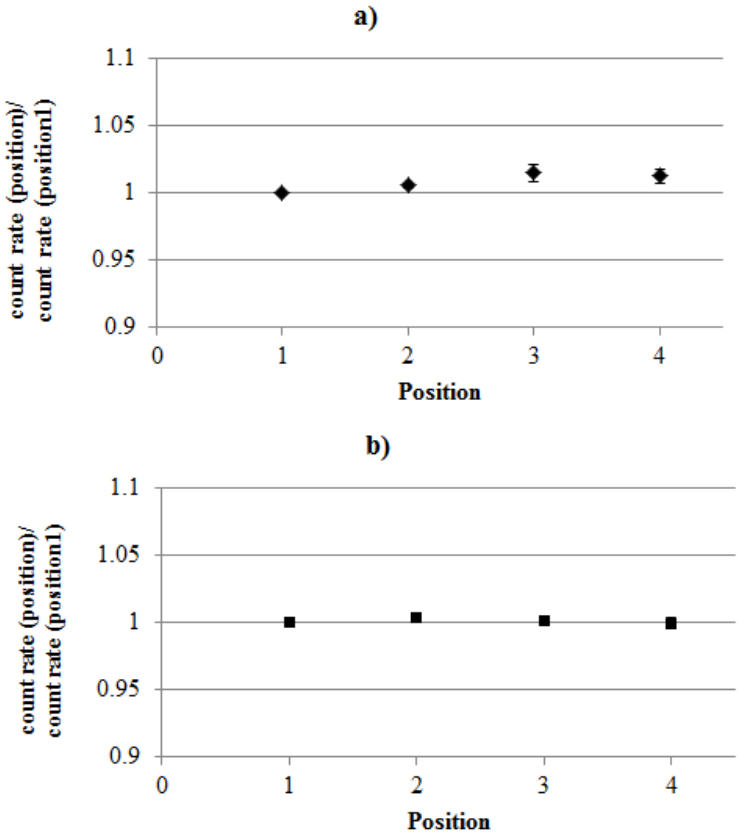

Figure 5: Investigation of geometric effect in measurements with gamma counter 1 (panel a) and 2 (panel b). The values are mean values of measured count rates of phantoms containing 25 kBq ^{131}I and nominal volumes of 0.05, 0.1, 0.5, 0.75 and 1 ml. The x-axis shows the positions according to Figure 1 and y-axis shows measured count rates given in relation to position 1. Error bars indicate SEM; error bars not visible are smaller than the data symbol

Biodistribution of ^{131}I

The biodistribution of ^{131}I was determined for 1 h up to 7 days after injection with injections performed at 12 am or 4 pm (Table 4; n=4-5/group). Some values are calculated for n=4 due to errors in the weight of the samples and s.c. injection. For injections performed at 12 am, the highest activity concentration, i.e. 2700 %IA/g, was observed in the thyroid at 18 h after injection. For injections performed at 4 pm, the highest concentration of activity was observed in the thyroid at 4 h after injection with 4500 %IA/g. High values were also found in gastric content, stomach, and salivary glands. It should be noted that about 6% of the data were not successfully measured with a minimum of 1000 counts over background due to low activity in respective samples. The ^{131}I activity in the total blood volume in the mice is presented as %IA in table 5. Statistical significant difference ($p=0.0023$) was observed 8 h after injection.

A compilation of measured ^{131}I activity in mouse organs as %IA/g for injections performed at 12 am is shown in Figure 6a. Results for respective gastric and intestine contents are presented in Figure 6c. Among organs, the highest activity concentration was observed in the thyroid for all time points following injection with a maximum after 18 h (2700 %IA/g). The heart depicted a maximum at 8 h, in the rest of the organs maximum values are observed at 1 h after injection. Highest %IA/g in declining order are given as follow; stomach, salivary gland, blood, lungs, small intestine, large intestine, kidneys, spleen, liver and heart. The highest value among contents was observed for the gastric content with 88 %IA/g 1 h after injection. Large intestine content had a maximum at 4 h (3.8 %IA/g) and small intestine content at 1 h (18 %IA/g).

Corresponding results for injections performed at 4 pm are shown in Figure 6b and Figure 6d. Among organs, the highest concentration of activity was observed in the thyroid at 4 h after injection for all time points following injection with a maximum after 4 h (4500 %IA/g). The rest of the organs showed maximum values after 1 h, highest %IA/g in declining order are given as follow; stomach, salivary gland, blood, lungs, small intestine, large intestine, kidneys, spleen, liver and heart. The highest value among the contents was observed in the gastric content at 1 h after injection with 246.87 %IA/g. For large and small intestine contents, a maximum of 3.63 and 6.11 %IA/g, respectively, were observed at 1 h after injection.

Table 4: Concentration of ^{131}I activity in mouse tissue at 1 h to 7 days after injection of 140–200 kBq at 12 am and 4 pm. Data are presented as mean (n=4-5) percent of injected activity per organ weight with SEM (italics)

Concentration (%IA/g)

Tissue	***1 h***		***4 h***		***8 h***		***18 h***		***24 h***		***3 days***		***7 days***	
Injection at 12 am														
Thyroid	720	*140*	1300	*240*	1100	*750*	2700	*550*	1100	*340*	470	*93*	210	*39*
Stomach	31	*3*	11	*2*	3.5	*0.5*	0.36	*0.07*	0.35	*0.05*	0.10	*0.02*	0.046	*0.002*
Blood	4.7	*0.4*	1.8	*0.2*	0.68	*0.08*	0.074	*0.012*	0.071	*0.011*	0.036	*0.005*	0.015	*0.001*
Small intestine	3.1	*0.6*	1.4	*0.3*	0.76	*0.07*	0.13	*0.02*	0.072	*0.017*	0.052	*0.008*	0.020	*0.002*
Kidneys	3.0	*0.1*	1.2	*0.0*	0.43	*0.04*	0.20	*0.01*	0.27	*0.02*	0.92	*0.09*	0.64	*0.04*
Large intestine	3.1	*0.3*	1.3	*0.1*	0.44	*0.05*	0.058	*0.007*	0.051	*0.007*	0.043	*0.005*	0.018	*0.002*
Salivary gland	11	*1*	10	*1*	4.3	*0.4*	0.26	*0.03*	0.22	*0.04*	0.074	*0.016*	0.022	*0.003*
Liver	2.0	*0.1*	0.71	*0.04*	0.33	*0.03*	0.10	*0.01*	0.10	*0.01*	0.071	*0.009*	0.032	*0.001*
Lungs	4.4	*0.3*	1.7	*0.1*	0.60	*0.07*	0.11	*0.01*	0.10	*0.01*	0.055	*0.006*	0.024	*0.001*
Spleen	3.0	*0.2*	1.1	*0.1*	0.48	*0.13*	0.058	*0.006*	0.048	*0.005*	0.025	*0.003*	0.011	*0.001*
Heart	0.0020	*0.0001*	0.00077	*0.0001*	0.28	*0.04*	0.044	*0.005*	0.039	*0.005*	0.027	*0.003*	0.010	*0.005*
Gastric contents	88	*23*	32	*5*	9.5	*0.9*	1.9	*1.1*	0.96	*0.06*	0.25	*0.03*	0.11	*0.01*
Large intestine contents	2.5	*0.3*	3.8	*0.3*	2.2	*0.4*	0.21	*0.02*	0.18	*0.02*	0.13	*0.01*	0.10	*0.03*
Small intestine contents	18	*10*	5.8	*2.7*	0.87	*0.15*	0.26	*0.06*	0.16	*0.02*	0.14	*0.04*	0.055	*0.012*
Injection at 4 pm														
Thyroid	1600	*830*	4500	*1800*	520	*220*	670	*210*	1300	*160*	450	*82*	200	*34*
Stomach	47	*3*	7.4	*1.7*	0.79	*0.16*	0.27	*0.03*	0.36	*0.08*	0.10	*0.02*	0.10	*0.03*
Blood	5.5	*0.6*	2.6	*0.5*	0.18	*0.02*	0.057	*0.006*	0.053	*0.010*	0.035	*0.004*	0.21	*0.17*
Small intestine	4.1	*0.4*	1.0	*0.2*	0.21	*0.02*	0.098	*0.018*	0.067	*0.006*	0.041	*0.004*	0.18	*0.06*
Kidneys	3.6	*0.1*	0.65	*0.06*	0.095	*0.009*	0.12	*0.02*	0.25	*0.03*	0.72	*0.07*	0.55	*0.06*
Large intestine	3.8	*0.6*	0.72	*0.16*	0.093	*0.016*	0.045	*0.005*	0.061	*0.014*	0.037	0.002	0.28	*0.13*
Salivary gland	16	*3*	5.5	*1.0*	0.67	*0.12*	0.21	*0.01*	0.20	*0.02*	0.059	*0.004*	0.12	*0.06*
Liver	2.4	*0.1*	0.44	*0.07*	0.085	*0.012*	0.072	*0.010*	0.10	*0.02*	0.079	*0.008*	0.083	*0.035*
Lung	5.3	*0.3*	1.0	*0.1*	0.17	*0.03*	0.085	*0.005*	0.071	*0.011*	0.047	*0.004*	0.10	*0.04*
Spleen	3.5	*0.2*	0.66	*0.09*	0.096	*0.010*	0.049	*0.004*	0.045	*0.005*	0.026	*0.003*	0.047	*0.023*
Heart	2.3	*0.1*	0.43	*0.06*	0.13	*0.05*	0.041	*0.007*	0.035	*0.005*	0.023	*0.002*	0.027	*0.011*
Gastric contents	260	*61*	18	*5*	0.11	*0.41*	9.5	*0.1*	1.9	*1.0*	0.96	*0.11*	0.25	*0.04*
Large intestine contents	3.6	*0.9*	2.5	*1.1*	0.29	*0.07*	0.17	*0.04*	0.18	*0.05*	0.34	*0.10*	0.093	*0.010*
Small intestine contents	6.1	*0.8*	1.2	*0.2*	0.19	*0.05*	0.12	*0.01*	0.11	*0.03*	0.10	*0.03*	0.50	*0.36*

Table 5: ^{131}I activity in total blood volume in mouse at 1 h to 7 days after injection of 140-200 kBq at 12 am and 4 pm.
Data are presented as mean (n=5) percent of injected activity with SEM (italics). Statistical significant difference was observed at 8 h (p=0.0023)

	%IA			
Time after injection	Injection at 12 am		Injection at 4 pm	
1 h	10	*1*	11	*1*
4 h	3.9	*0.3*	5.4	*1.1*
8 h	1.4	*0.2*	0.37	*0.04*
18 h	0.16	*0.02*	0.12	*0.01*
24 h	0.15	*0.03*	0.11	*0.02*
3 days	0.076	*0.010*	0.070	*0.007*
7 days	0.030	*0.002*	0.42	*0.34*

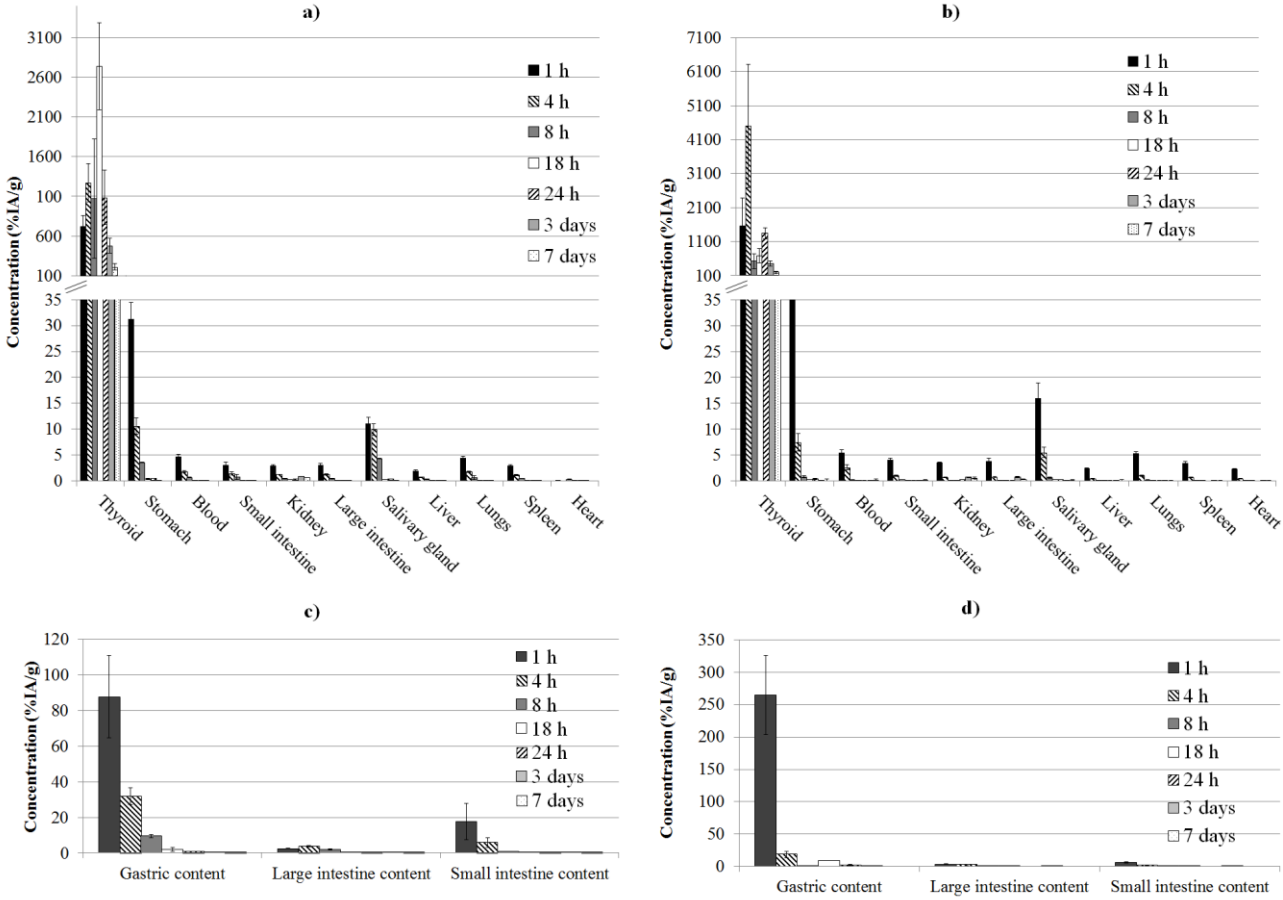

Figure 6: Biodistribution of ^{131}I in mouse at 1 hour to 7 days after injection of 140–200 kBq performed at 12 am (panel a and c) and 4 pm (panel b and d). Concentration is given as mean percent of injected activity per organ weight (%IA/g), corrected for physical decay to the time of injection. Error bars indicate SEM. Note the change of scale on the y-axis.

Biokinetics of ^{131}I for injections performed at 12 am or 4 pm are presented for each sample in Figures 7-20. The p-values obtained from Student's t-test are shown for those time points where a statistically significant difference between %IA/g at 12 am or 4 pm was observed. Statistically significant difference was observed at least one time point in all samples, mostly at 4 and 8 h after injection.

In the thyroid, see Figure 7, a statistically significant difference ($p=0.016$) was observed at 18 h after injection. Furthermore, the activity concentration reached a maximum at different time points after injection, i.e. after 18 h for injection at 12 am and after 4 h for injection at 4 pm. The maximum at 18 h for injections performed at 12 am is statistically significantly different ($p=0.039$) from the value at 24 h. For injections performed at 4 pm, a local maximum is observed at 24 h with statistically significant difference from the values at 18 h ($p=0.036$) and 3 days ($p=0.0025$). Large deviations from the mean were observed for values up to 24 h after injection, but not at later time points. In the stomach (see Figure 8), blood (see Figure 9), lungs (see Figure 15), salivary gland (see Figure 13), spleen (see Figure 16) and liver (see Figure 14), the activity concentration had an exponential decrease and statistically significant differences were observed for one or two time points. In small intestine, see Figure 10, statistically significant differences were observed at 8 h and 7 days after injection. While the activity concentration showed a monotonous decline for injections at 12 am, a slight increase in %IA/g at 7 days was observed.

The activity concentration in the kidneys, see Figure 11, did not depict a monotonous decline: the global maximum was observed at 1 h after injection followed by rapid decrease; a small increase was observed again after 18 h with a local maximum at 3 days. The difference between the concentrations observed at 24 h and at 3 days are statistically significant for both injection series, $p<0.0001$. Statistically significant differences between the injection series were observed at 1, 4, 8 and 18 h after injection. For the large intestine, see Figure 12, statistically significant differences were observed at 4 and 8 h after injection. While biodistribution kinetics showed a monotonous decline for injections at 12 am, a slight increase in %IA/g after 24 h was observed for injections at 4 pm with a local maximum around 3 days after injection. The observed concentration at 3 day is significantly different from concentration at 24 h ($p=0.0029$) and at 7 days ($p=0.031$).

In the heart, see Figure 17, statistically significant differences were observed at 1, 4 and 8 h, with comparatively large differences in %IA/g at 1 and 4 h. The concentration of the activity reached a maximum at different time points depending on time of injection, i.e. after 8 h for injections at 12 am and after 1 h for injections at 4 pm.

The measured activity for the gastric content, see Figure 18, showed statistically significant difference at 8 h; large deviation from the mean was observed for injections at 4 pm. For the small intestine content, see Figure 19, statistically significant difference was also observed at the same time point (8 h); large deviation from the mean at early time points was also observed for injections at 12 am. Likewise, for the large intestine content, see Figure 20, statistically significant difference was also observed at 8 h; large deviation from mean was observed for values up to about 20 h.

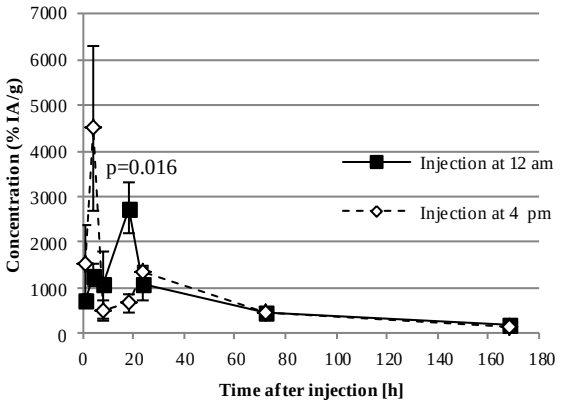

Figure 7: Concentration of ^{131}I in the thyroid with injections performed at 12 am or 4 pm. Statistically significant difference was observed at 18 h (p=0.016). Error bars indicate SEM, n=5

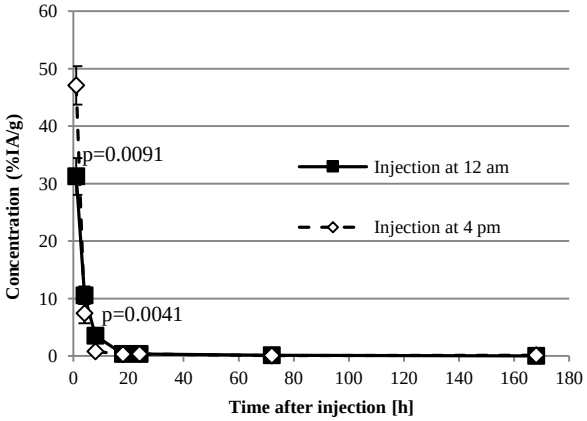

Figure 8: Concentration of ^{131}I in the stomach with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 1 h (p=0.0091) and 8 h (p=0.0041). Error bars indicate SEM, n=5

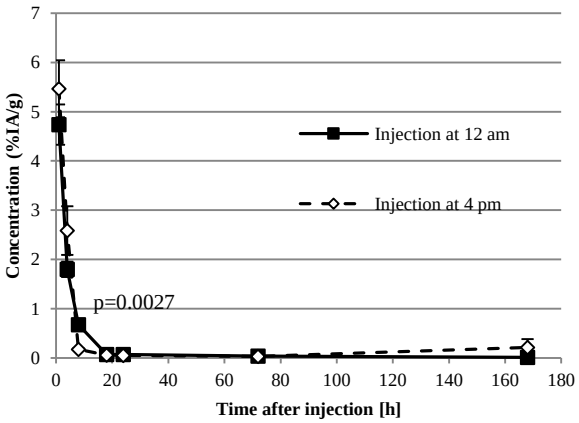

Figure 9: Concentration of ^{131}I in blood with injections performed at 12 am or 4 pm. Statistically significant difference was observed at 8 h (p=0.0027). Error bars indicate SEM, n=5

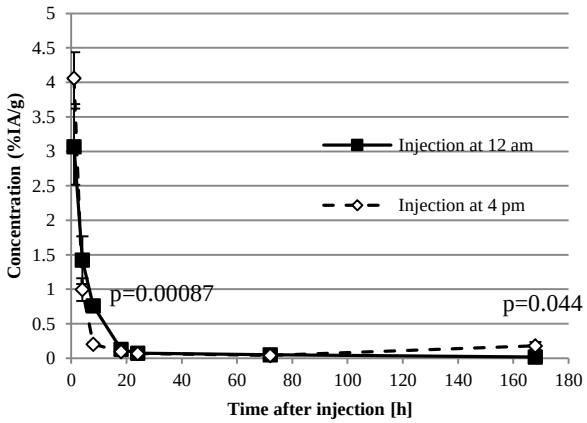

Figure 10: Concentration of ^{131}I in the small intestine with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 8 h (p=0.00087) and 7 days (p=0.044). Error bars indicate SEM, n=5

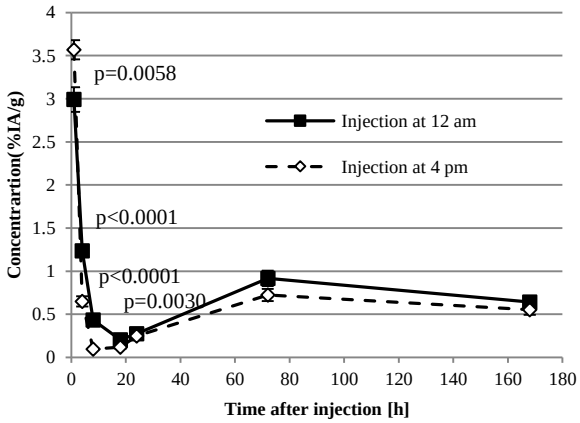

Figure 11: Concentration of ^{131}I in the kidneys with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 1 h (p=0.0058), 4 h (p<0.0001), 8 h (p<0.0001) and 18 h (p=0.0030). Error bars indicate SEM, n=5

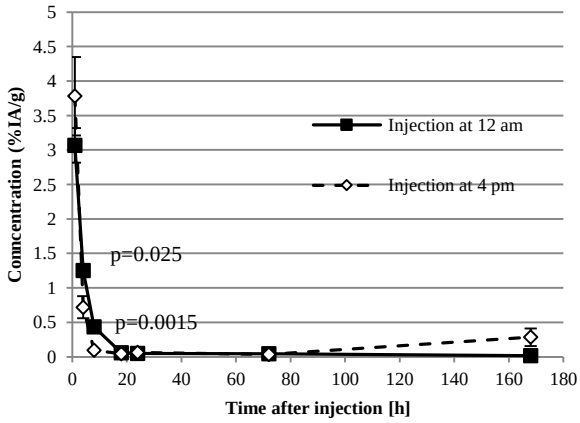

Figure 12: Concentration of ^{131}I in large intestine with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 4 h (p=0.025) and 8 h (p=0.0015). Error bars indicate SEM, n=5; note exception (n=4) for group at 1 h injected at 4 pm

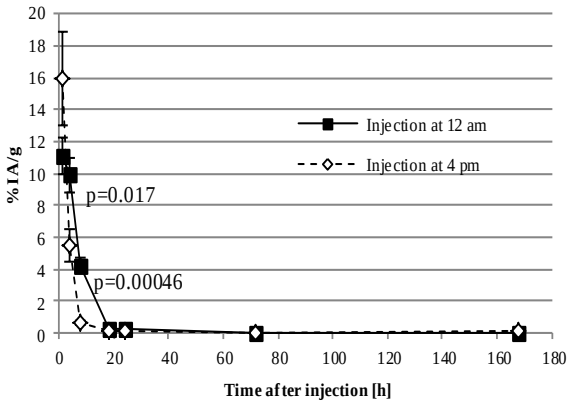

Figure 13: Concentration of ^{131}I in salivary gland with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 4 h (p=0.017) and 8 h (p=0.00046). Error bars indicate SEM, n=5

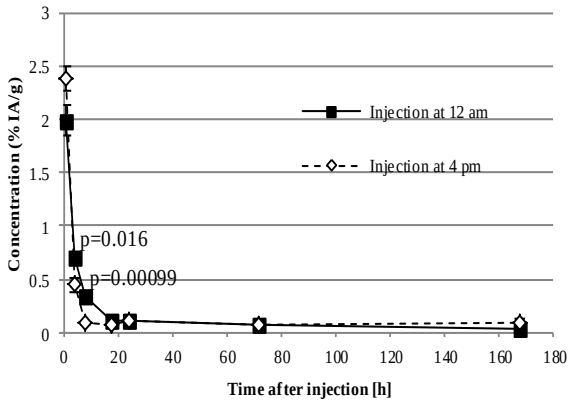

Figure 14: Concentration of ^{131}I in liver with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 4 h (p=0.016) and 8 h (p=0.00099). Error bars indicate SEM, n=5

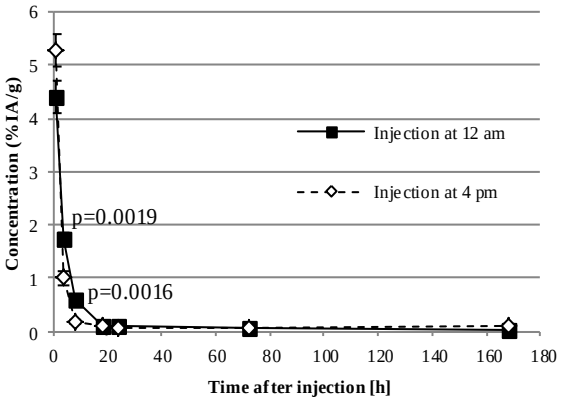

Figure 15: Concentration of ^{131}I in lungs with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 4 h (p=0.0019) and 8 h (p=0.0016). Error bars indicate SEM, n=5

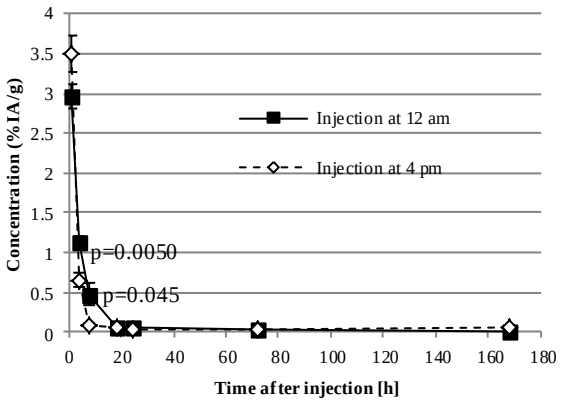

Figure 16: Concentration of ^{131}I in spleen with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 4 h (p=0.0050) and 8 h (p=0.045). Error bars indicate SEM, n=5

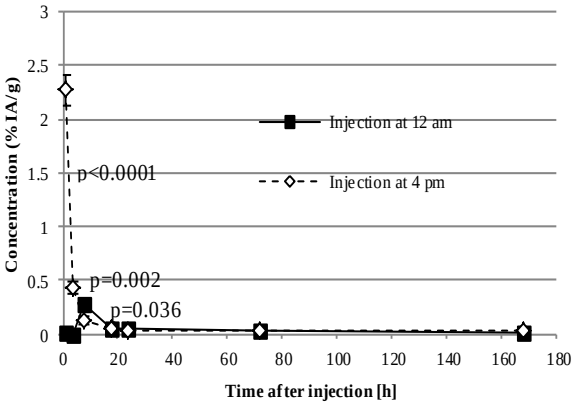

Figure 17: Concentration of ^{131}I in heart with injections performed at 12 am or 4 pm. Statistically significant differences were observed at 1 h (p<0.0001), 4 h (p=0.002) and 8 h (p=0.036). Error bars indicate SEM, n=5

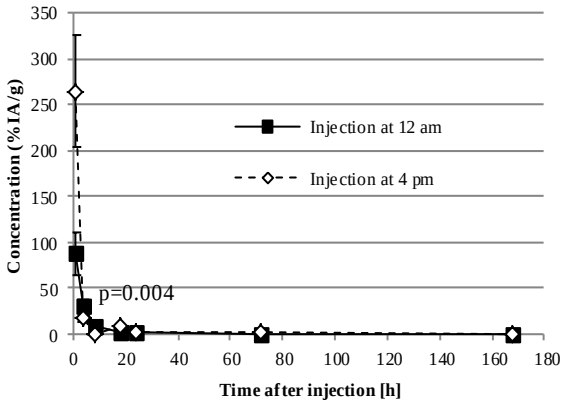

Figure 18: Concentration of ^{131}I in gastric content with injections performed time at 12 am or 4 pm. Statistically significant difference is observed at 8 h (p=0.004). Error bars indicate SEM, n=5 except for 3 days injection at 4 pm which has n=4

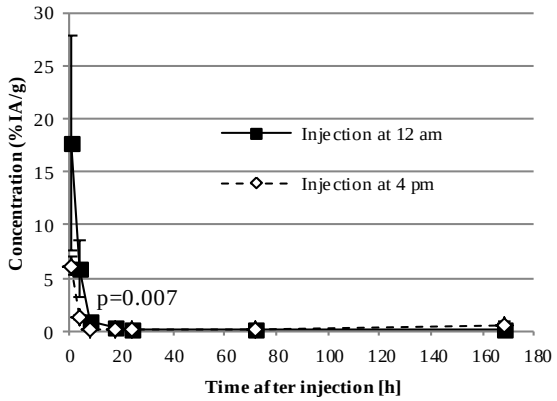

Figure 19: Concentration of ^{131}I in small intestine content with injection performed at 12 am or 4 pm. Significant differences were observed at 8 h (p=0.007). Error bars indicate SEM, n=5; note exception (n=4) for group at 4 h injected at 12 am

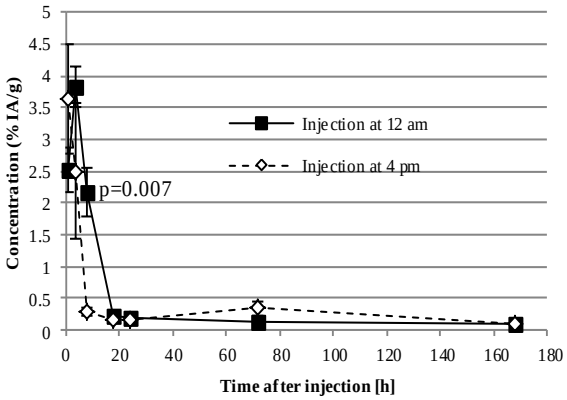

Figure 20: Concentration of ^{131}I in large intestine content with injections performed at 12 am or 4 pm. Statistically significant difference is observed at 8 h (p=0.007). Error bars indicate SEM, n=5

Discussion

Animal experiments were performed to investigate the potential effect of circadian rhythm on the biodistribution and biodistribution over time of ^{131}I in mice. The activity concentration was studied in various organs at different time points after radionuclide administration. The effect of circadian rhythm was studied by performing ^{131}I administrations at different times of day. In order to minimize the errors in the gamma counter measurements, investigation of self-attenuation in the sample and potential geometric effects were performed, together with appropriate calibration of the detector.

The calibration factors for ^{131}I were obtained by a linear fit to the measured count rates up to about 11 000 cps for gamma counter 1 and 14 000 cps for gamma counter 2 *vs*. the activity in the samples. The obtained R^2-values of 0.9995 showed that the linear curve fitting was a good approximation. The linearity of the relationship indicates that the automatic correction for dead time by the gamma counter can be accepted up to this point (dead time factor up to 12% for gamma counter 1 and 15% for gamma counter 2).

The result from the investigation of self-attenuation of ^{131}I in tissue equivalent phantoms with constant activity concentration showed a linear relationship between the measured count rates and volume of the phantoms (and thus ^{131}I activity). The R^2-value of 0.9977 showed that the linear fit was a good approximation which means that no volume effect (self-attenuation) was observed.

The preparation of tissue equivalent phantoms with similar amount of ^{131}I activity contained some uncertainties. There were errors in the uniformity of the activity distribution in the gel stock solutions resulting in uncertainties of the activity in the phantoms. Other errors concerned the density of the gel and volume of the phantoms. It should be noted that phantom mass was lower than expected, which indicated loss of gel in the preparation. This means that the volumes of the phantoms were lower than nominally specified. Due to these uncertainties, a rigorous statistical analysis was performed accepting significant difference with $p \leq 0.01$. The large SEM for the 0.05 and 0.1 ml phantoms can be explained by the difficulties in preparation of these small volumes. The analysis showed no statistically significant difference between the measured cps, and hence, no correction for volume effects was necessary in the animal experiments.

The investigation of a potential geometric effect in gamma counter measurements showed no statistically significant difference between cps measured for the four different orientations of the phantoms. A central position in the 20 ml scintillation vial was not investigated due to the concave bottom of the vial, which results in sliding of samples to the edge of the vial. No geometric effect was observed meaning that the position of the organ sample can be arbitrary as long as it is located at the edge of the scintillation vial.

The animal study indicated that the biodistribution of ^{131}I in mice is influenced by the time of day when the radionuclide is administered. In the kidneys, large intestine and heart, statistically significant differences in concentration of ^{131}I activity were detected at three or four time points after administration. In the other investigated organs and tissues, statistically significant differences were observed for one or two time points. Most of the differences were observed at 4 h and/or 8 h after administration and occasionally at 1 h, 18 h, 3 days, or 7 days. The difference in activity concentration between the two injection series is thought to be a consequence of differences in biological processes as a result of circadian rhythm.

The concentration of ^{131}I in the thyroid showed different behavior over time between the two injection series. Maximum values were observed at different time points; 18 h for injections preformed at 12 am or 4 h for injections performed at 4 pm. Also a local maximum was observed at 24 h for injection 4 pm. These differences could result in different absorbed dose in the tissue. Nevertheless, statistically significant difference between two administration series was only observed at one time after injection. Why statistically significant difference was not detected more often in this case is probably due to the large SEM in the thyroid values. Walinder studied the difference in ^{131}I concentration in the thyroid after 24 h for injections performed at 8 am, 1 pm or 4 pm (Walinder, 1971). That study demonstrated higher ^{131}I concentration in thyroid for injection at 1 pm compared with 4 pm. When comparing data at 24 h from the present study, the ^{131}I concentration in thyroid did not differ between the groups (12 am and 4 pm), which is in contrast with the Walinder study. However, the differences seen at 18 h in the present study is in line with the findings by Walinder, and could be explained by differences in metabolic activity between the animals in the two studies.

The large uncertainties observed in the ^{131}I concentration in thyroid could partly be explained by difficulties in the dissection. In most samples, the actual tissue weight was used, assuming only thyroid tissue in the sample. However, 26 of the thyroid samples had unreasonably high mass (deviating more than 1 SD from supposed mean value), which indicates that the samples did not only contain thyroid tissue, but also surrounding tissue: within about 24 h after injection, the ^{131}I concentration in the blood was high and the perfusion of surrounding tissue in the throat would lead to a significant amount of ^{131}I in the tissue. Consequently, ^{131}I in surrounding tissue would then contribute to the measured %IA/g of imperfectly dissected thyroid samples. This could explain why the observed SEM values in the ^{131}I concentration were larger for the time points up to 24 h, compared with later time point when the ^{131}I concentration in the surrounding tissue should be insignificant. Hence, an average thyroid mass value was used for those samples, as calculated from samples with reasonable mass based on literature. Furthermore, four of the animals, found in the group killed 8 h after injection performed at 12 am and the groups killed 1, 8 and 18 h after injection performed at 4 pm, showed very low values of activity concentration which contributed to large deviation. In contrast, these animals did not show such divergent values in other organs or gastric and intestine content. Large uncertainties in thyroid concentration of radioiodine has previously been shown and has not been explained by other factors than differences in stable iodine intake and individual metabolic differences (Lundh *et al*., 2006, Spetz *et al*., 2013) .

The concentration of iodine in the food given to the animals affects the uptake of the radioactive iodine in the thyroid (Walinder, 1971). High amount of accumulated stable iodine blocks the uptake of radioactive iodine. Walinder used animal diets with different iodine concentrations from 0.33 to 0.74 µg/g. The study demonstrated that differences in ^{131}I uptake in the thyroid for the different times of administration were not influenced by the iodine concentration in the food although the uptake was lower for the animals that were given food with higher iodine content. The animals in the present study were given breeding food with a reduced iodine concentration of 0.87 µg/g compared with 1.202 µg/g in regular food. Nevertheless, the relatively high iodine content in breeding food, as compared with the iodine-reduced food used by Walinder, may have resulted in lower ^{131}I concentration in the thyroid. In order to minimize the reduction in uptake of radioactive iodine, the mice should be on an even lower iodine concentration diet for a period of time before and after the ^{131}I administration. Another important aspect is free access to food. Food *ad libitum* is standard procedure in care of laboratory animals, but it makes it difficult to monitor when and how much each individual mouse eats. The individual food intake may cause a greater deviation around the mean uptake in the thyroid or other organs in an animal group,

which may impede demonstration of statistically significant differences between values. The access of food should be controlled in future studies in order to minimize this deviation.

The concentration in the kidneys had a global maximum at 1 h after injection and a local maximum at 3 days. A local maximum is also found in the large intestine. The ^{131}I concentration in the heart showed different curve behavior between the two administration time points. The maximum values are observed at different time points and statistically significant differences in %IA/g were found at time points up to 20 h after injection. The %IA/g in the gastric content as well as the in large and small intestine contents showed relatively high values. This finding was unexpected, since ^{131}I was administered intravenously and not orally. However, ingestion of ^{131}I may be possible through urine absorbed in the nesting material in the cages. A likely explanation is that the mice had eaten of the nesting material and thereby received activity orally as well. This may also explain the large SEM since the amount and timing of ingestion this is an effect of individual behavior in animals. The amount of gastro-intestinal contents varied greatly between groups and between mice in the same group, which contributed further to large SEM values. Nevertheless, from the data obtained in this study, it is not possible to exclude the possibility of a potential secretion mechanism for iodide into gastric (or intestinal) content, even if considered unlikely to this extent.

The administrated ^{131}I activity of 150 kBq was selected to provide a sufficiently high activity for analysis without stunning the thyroid (Postgård, *et al*., 2002, Lundh, *et al*., 2009). The majority of the samples contained very low activity; hence, not all samples achieved a minimum 1000 counts over background. At the time of this report, these samples are re-measured for future work.

The mouse is a nocturnal (night active) animal with an inverted circadian rhythm compared with humans. The injections were performed at two times of day with a time difference of 4 h, i.e. 12 am and 4 pm. The time points were selected to reflect normal laboratory work hours, which for the mouse coincides with the time when the animal is least active. It is reasonable to assume that a larger time difference between the day times of injection should improve the ability to observe statistically significant difference in %IA/g, i.e. to demonstrate dependence of circadian rhythm for more time points and tissues. For instance, the impact of circadian rhythm on biodistribution (or other biological endpoints) may be more apparent if the injection series were to be performed at e.g. noon and at midnight.

Conclusion

Measurement corrections for sample volume regarding self-attenuation or geometric effects in the gamma counter were not necessary.

The results from the animal experiment showed difference in biodistribution and biokinetics in dependence of time of ^{131}I administration. The concentration in the thyroid showed different behavior over time between the two injection series (injections at 12 am and 4 pm). A statistically significant difference was observed at 18 h and the two groups also seemed to have their maxima at different time points after injection. This may have an effect on the absorbed dose in the organ and should be investigated in future work.

This study indicated that circadian rhythm influences the ^{131}I biodistribution over time and strongly suggests that this phenomenon should be considered in absorbed dose calculations for ^{131}I. Moreover, these findings advocate that, in the future, time of day should be considered as a variable in biodistribution data. Accordingly, biodistribution data should be used with regard to when administrations were performed during the day.

The effects of circadian rhythm on biodistribution and biokinetics of ^{131}I in humans need to be investigated. The result from this work suggests that therapeutic administration of ^{131}I in clinical use can be further optimized based on circadian rhythm. With knowledge of how the biodistribution varies with time of day of administration an optimum time-point can be chosen. For treatment this could mean a time-point resulting in higher absorbed dose to the target without influencing the absorbed dose to organs at risk, or the same absorbed dose to the target and lower absorbed dose to surrounding organs.

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