Estimating the absorption of soil-derived uranium in humans

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ABSTRACT

The aim of the present study was to improve the estimation of soil-derived uranium absorption in humans. For this purpose, an *in vitro* solubility assay was combined with a human study by using a specific edible soil low in uranium. The mean bioaccessibility of the soil-derived uranium,

determined by the solubility assay in artificial gastrointestinal fluid, was found to be 7.7% with a standard deviation of 0.2%. The corresponding bioavailability of the soil-derived uranium in humans was assumed to be log-normal distributed with a geometric mean of 0.04% and a 95% confidence interval ranging from 0.0049% to 0.34%. Both results were used to calculate a factor, denoted as f_A^{sol}, which describes the relation between the bioaccessibility and the bioavailability of soil-derived uranium. The geometric mean of f_A^{sol} was determined to be 0.53% with a 95% confidence interval ranging from 0.06% to 4.43%. Based on f_A^{sol}, it is possible to estimate more realistic values on the bioavailability of uranium for highly uranium-contaminated soils in humans by just performing the applied solubility assay. The results of this study can be further used to obtain more reliable results on the internal dose assessment of ingested highly uranium-contaminated soils.

INTRODUCTION

The aim of the present study was to improve the estimation of soil-derived uranium absorption in humans.

Ingestion of small amounts of soil by humans can be observed around the world. It occurs involuntarily or deliberately. Involuntary routes of soil ingestion are via inhalation and swallowing of dust, via food and via hand-to-mouth activities of young children. Deliberate ingestion of soils, which is also called geophagy, is reported, e.g. in Africa, Asia and South America.[1, 2] In European countries like Germany some people also practice geophagy by eating soils called healing soil or medical soil.[3] These soils can be purchased without a prescription and are used to cure moderate alimentary tract related symptoms like acid reflux.

Recent values concerning the amount of soils ingested by children are varying from 26 mg/day to 100 mg/day.[4, 5] The average soil ingestion by adults is assumed to be 10 mg/day, whereas

deliberate ingestion of soils like healing soil can amount from 6 g/day to 40 g/day over several weeks.[3, 6]

Uranium, as a naturally occurring radioactive material (NORM), occurs ubiquitously in soils at concentrations of about 3 mg/kg and comprises the radio-isotopes ^{234}U, ^{235}U and ^{238}U with percentages by mole fraction of 0.0054%, 0.72% and 99.27%, respectively.[7, 8] In some areas, the amount of uranium in soils is technologically enhanced (TENORM) e.g. due to uranium refining, agriculture activities and presumably also as a result of nuclear incidents like the Chernobyl accident in 1986 or the Fukushima Daiichi incident in 2011.[9-11] Consequently, soil ingestion leads to a certain internal dose enhancement.

The internal dose caused by ingested uranium is calculated by using the current biokinetic compartmental model reported by the International Commission on Radiological Protection (ICRP).[12] This biokinetic model describes the uptake, distribution and deposition of uranium in tissues and its excretion from the human body. One important parameter within this model is the so-called f_1 value, which quantifies the bioavailability of uranium. The bioavailability (f_1) of uranium is defined as the fraction of ingested uranium which is absorbed from the alimentary tract into the circulatory system.[13]

It is noted, however, that the f_1 value of uranium was established by the ICRP for ingestion scenarios of soluble uranium, especially considering the uptake of uranium from drinking water.[12] To estimate the bioavailability (f_1) of uranium from a highly uranium-contaminated soil, human soil ingestion studies e. g. of soils from uranium mining sites are inappropriate. Instead, *in vitro* solubility assays, which mimic the alimentary tract conditions, are applied. By these assays only the bioaccessibility but not the bioavailability of uranium can directly be estimated. The bioaccessibility (DF) of uranium is defined as the fraction of uranium, which is soluble in

64 the alimentary tract and therefore potentially available for absorption.[13] To derive the
65 bioavailability (f_1) of uranium from the bioaccessibility (DF) data, some investigators assumed
66 that the total fraction of bioaccessible uranium is absorbed into the circulatory system, hence
67 equating bioavailability with bioaccessibility.[14] Others estimated a proportion of only 0.2% - 5%
68 of bioaccessible uranium to be absorbed by the alimentary tract into the blood.[3] As a
69 consequence, these differences ended up in a high variation of the estimates of the f_1 value for
70 uranium of up to three orders of magnitude.

71 Additionally, different established solubility assays like the solubility assay "DIN 19738" and
72 the “US P”- method showed varying estimates of the bioaccessibility for uranium despite using
73 an identical soil.[3] These differences in the bioaccessibility data are expected to be the result of
74 the different composition of those assays, which further increases the uncertainty of the
75 bioaccessibility and consequentially the uncertainty of the estimated bioavailability.

76 For the reasons mentioned above, the aim of this study was to set up a method which enables
77 the assessment of more reliable data on the bioavailability for soil-derived uranium. The idea
78 was to combine a solubility assay with a human study by using an edible healing soil as a model
79 soil for both investigations. Thereby, the bioaccessibility value and the corresponding
80 bioavailability value of the healing soil-derived uranium can be obtained experimentally. Both
81 values can then be used to calculate a factor, which describes the relation between the
82 bioaccessibility and the bioavailability of soil-derived uranium.

83 This factor can be further used to obtain more realistic data on the bioavailability of uranium
84 dissolved from various soils by just performing a solubility assay. Thereby, especially highly
85 uranium contaminated soils and potentially also industrial forms of uranium can easily be

86 investigated to determine realistic bioavailabilities. Finally, more reliable data on the internal
87 dose due to soil ingestion can be obtained.

EXPERIMENTAL SECTION

Experimental design and calculation of f_A^{sol}

The notation of the bioavailability has changed from f_1 to f_A by the ICRP.[15] Therefore, the results on bioavailability obtained in this study are denoted as f_A, while data on the bioavailability from previous studies are still denoted as f_1.

The present work was designed to determine a factor, denoted as f_A^{sol}, which connects the bioaccessibility DF and the bioavailability f_A for soil-derived uranium. This factor (f_A^{sol}) is defined as the fraction of bioaccessible uranium, which is absorbed from the alimentary tract into the circulatory system. It can be determined as previously published by:[3]

$$f_A^{sol} = \frac{f_A}{DF} \qquad (1)$$

Hence, f_A^{sol} can be derived by using the same edible soil for a solubility assay and a human study to evaluate DF and f_A, respectively. The relation of the bioavailability (f_A), the bioaccessibility (DF) and the factor f_A^{sol} is depicted in Figure 1.

Healing soil

In the present study, the healing soil "Luvos®Heilerde ultrafein" (Luvos Just GmbH&Co, Friedrichsdorf, Germany) was selected as the model soil for the solubility assay and the human study because it fulfills all necessary requirements. An important aspect here is that the healing soil "Luvos®Heilerde ultrafein" is a medical product which is suitable for ingestion by humans. It can easily be purchased in sufficient quantities without a prescription and is ultra-fine (<1 µm) and therefore homogeneous, which ensures a constant quality during the whole study. The healing soil "Luvos®Heilerde ultrafein" contains a small concentration of the naturally occurring radionuclide ^{238}U.[3]

Determination of ^{238}U in healing soil

For the analyses of total ^{238}U in healing soil 139.2 mg of healing soil were mixed with 5 mL of HNO_3 (65%), 1 mL of HCl (30%) and 1 ml HF (40%). The mixture was microwave-assisted digested in a Multiwave 3000 (Anton Paar, Austria); power: ramp for 15 min up to 1400 W, hold for 30 min at 1400 W, cooling down for 15 min. After the subsequent addition of 6 mL of H_3BO_3 to neutralize free fluorides the solution was placed again in the Multiwave system, applying a 5 min ramp up to 900 W, hold for 15 min and cooling down for 15 min. Thereafter, the solution was stored at 4 °C until measurement of ^{238}U using ICP-MS (Supporting Information, Section S.1).

Gastric pH-metry – Determination of the gastric pH

To ensure that the solubility assay DIN 19738 (see below) realistically reproduces the gastric pH-conditions and therefore presumably provides more realistic data on the bioaccessibility for healing soil-derived uranium, the *in vivo* gastric pH after soil ingestion had to be determined. For this purpose, a volunteer test with a 29-year-old male was performed where the gastric pH status after healing soil ingestion was measured by means of a standard gastric pH-metry. After an overnight fast, a pH glass probe (F8/IR BLUE LINE, SMT Simtec GmbH, Switzerland) was inserted via nose, throat, and esophagus into the stomach. This was carried out under medical supervision in the clinic Rechts der Isar, TU München, Germany. The probe was connected during the whole experiment to a portable recorder (DL 70, Standard Instruments GmbH, Germany). The setup enabled the volunteer to eat, drink and act as usual. One hour after probe insertion, 20 g of healing soil slurried in 400 mL water (MilliQ) were quickly ingested, according to the standard healing soil ingestion protocol. The pH data were recorded during the whole experiment and noted as “physiological”. However, the pH-metry data were only available as raster graphics image and therefore redigitized by using the WinDIG 2.5 program.[16] Thereby

mean pH values over about 1.5 min were obtained with estimated standard uncertainties of the mean pH values (U_m) of 0.2.

Solubility assay – Determination of DF

To determine the bioaccessibility of uranium within the alimentary tract, an *in vitro* solubility assay was performed based on the German method DIN 19738.[17] This solubility assay included artificial gastric and intestinal fluids composed of organic and inorganic compounds. The composition of these fluids was already presented elsewhere.[3] Incubation times, temperature and partly pH values were also adapted from the DIN 19738. As the absorption of uranium from the gastrointestinal tract into the systemic circulation occurs mainly in the small intestine,[18] only the entire incubation mixture (gastric and intestinal) was used for the quantification of soluble ^{238}U.

The *in vitro* solubility experiments were performed by incubating 2 g of healing soil in 100 mL of artificial gastric fluid for 2 h using a pH status of 1.0 ± 0.2, 2.0 ± 0.2 or the "physiological" pH-status as assessed in the investigation described in the previous section. The pH status was adjusted with HCl (10%). The suspension was held at 37 °C ± 1 °C and was constantly stirred at about 500 rpm to avoid sedimentation. Thereafter, 100 mL of artificial intestinal fluid were added and the pH was adjusted to 7.5 ± 0.2 and held by the addition of $NaHCO_3$ and HCl, respectively. The suspension was stirred for another 6 h at 37 °C ± 1 °C. After a total of 8 h, 50 mL-fractions were taken and centrifuged at 5000 rpm (Hettich Universal 32R). Three aliquots of 10 mL were taken. From these samples, two aliquots were further filtered at 0.45 µm or 0.2 µm (sterile filter, Millipore). A fourth sample was directly taken from the suspension and filtered at 0.2 µm without centrifugation. For each solubility experiments, extractions were prepared in triplicates. To correct for the background of ^{238}U the solubility assays were run without soil. The

156 samples were stored at 4 °C until measurements of ^{238}U were performed using an inductively
157 coupled plasma mass spectrometer (ICP-MS) (Supporting Information, Section S.1).

158 Human Study – Determination of f_A

159 The human study was conducted on healthy volunteers according to the principles of the
160 Declaration of Helsinki, under the ethical authorization of the competent review boards
161 (Technical University Munich, Germany, Ethical Commission), and with patients' written
162 consent.

163 Ten volunteers (6 females, 4 males, aged 22-55 years) without chronic disorders participated in
164 the human study, which was performed between 2011 and 2013. Nine volunteers participated
165 once. One male volunteer (Volunteer 1) participated three times. These three investigations were
166 done to investigate any possible individual variations. Only averaged results of all three
167 investigations were used for further calculations. Therefore, every volunteer is equally
168 represented in the current study.

169 All volunteers had to collect their complete 24-h-urine over three days before (day 1, day 2,
170 day 3) and three days after (day 4, day 5, day 6) a single ingestion of 20 g of healing soil mixed
171 in 400 mL water (MilliQ). The soil ingestion was at day 4 around 8:00 a.m. after a 10 h
172 overnight fast. After 2.5 hours the volunteers were provided with a standard breakfast (bread roll,
173 jam, and tea, water or coffee). Afterwards, the volunteers were allowed to eat and drink ad
174 libitum.

175 The amounts of applied healing soil were not considered to pose any health risk to the human
176 volunteers. The volunteers were never knowingly exposed through ingestion or inhalation to any
177 other increased levels of uranium. All participants were provided with polyethylene bottles of 3

178 L capacity (Sarstedt, Nümbrecht, Germany) and were given instructions on how to collect their
179 urine.

180 The total weights of all 24-h-urine samples were recorded. Subsamples of 20 mL were drawn,
181 acidified with subboiled distilled nitric acid in a sterile polystyrene test tube and stored frozen at
182 −20°C until analysis by ICP-MS (Supporting Information, Section S.2). Daily urine volumes
183 were calculated by dividing the weighed urine masses by the urine specific density factor of
184 1.020 g/mL ± 0.015 g/mL.[19]

185 To determine the bioavailability (f_A) of soil-derived ^{238}U for the human volunteers, the current
186 systemic biokinetic compartmental model for uranium published by the ICRP[12] together with the
187 measured urinary excretion data of ^{238}U were used.

188 Total daily ^{238}U excretion of volunteers was calculated by multiplying the concentrations of
189 ^{238}U in the 24-h-urine samples with the corresponding urine volumes. To correct for the
190 background of dietary uranium excretion total healing soil-derived ^{238}U excretion (m_{te}) was
191 estimated for a period of 3 days after soil ingestion by $m_{te} = m_a - m_b$, where m_a is the
192 cumulated amount of excreted ^{238}U over 3 days after soil ingestion and m_b is the cumulated
193 amount of excreted ^{238}U obtained over 3 days before soil ingestion. According to the current
194 biokinetic compartmental model for uranium, the urinary systemic excretion of absorbed
195 uranium over 3 days amounts to 68.5% in total.[20] Therefore, the total amount (100%) of
196 absorbed soil-derived uranium (m_{ta}) was calculated by $m_{ta} = m_{te} \times 100/68.5$.

197 Finally, based on the total amount of ingested ^{238}U from 20 g of healing soil (m_{ti}) f_A was
198 calculated by:

199 $$f_A = \frac{m_{ta}}{m_{ti}} \qquad (2)$$

The applied 68.5% of urinary excreted uranium over three days does not account for the slight delay of uranium excretion due to the transition time of healing soil in the upper gastrointestinal tract after ingestion. However the conducted urinary sample collection over three days is sufficiently long to compensate for this delay.

RESULTS AND DISCUSSION

Gastric pH-metry – Determination of the gastric pH

In accordance with the physiological conditions of the alimentary tract, the procedure of the solubility assay DIN 19738 recommends a sequential incubation of soil in artificial gastric and intestinal fluid with a pH value of 2 over 2 hours and a pH value of 7.5 over 6 hours, respectively.

However, these standard conditions may not be valid for the specific case of ingestion of healing soil, since one characteristics of the healing soil in humans is its capability to neutralize gastric acid after ingestion. Moreover, preliminary results of the solubility assay DIN 19738 had shown that the gastric pH-value is a sensitive parameter when assessing the solubility of healing soil-derived ^{238}U (a 60% decrease in solubility was observed when varying the pH-value from 2 to 4; data not shown). Yet, it remained unknown to which extent the ingested healing soil was actually capable to neutralize the real gastric acid. Therefore, it was important to find out the *in vivo* gastric pH status after healing soil ingestion. For this reason, a standard gastric pH-metry was done on a 29-year-old male volunteer while ingesting the healing soil. The gastric pH status was recorded for the initial two hours after soil ingestion (Figure 2).

After an overnight fast, the pH value of the volunteer was about 1.0 ± 0.2. After healing soil ingestion, the gastric pH-metry revealed a rapid increase of the gastric pH up to 5.1 within 6 min, followed by a slow decline. About 30 min after soil ingestion, the gastric pH was at 2.0 and further declined towards pH 1.0 within the next 60 min. For the remaining 30 min, the gastric pH level stayed at about 1.0 ± 0.2. From these data gastric pH values of 1.6 (mean) and 1.3 (median) can be evaluated.

Solubility assay – Determination of DF

As a conclusion from the gastric pH-metry, two alternative gastric pH courses were applied in addition to the recommended stable gastric pH of 2 for the solubility assay DIN 19738, while all other parameters of the solubility assay were not altered. The first alternative gastric pH course was directly adopted from the gastric pH-metry. In this case, the complete pH pattern over 2 hours, shown in Figure 2, was mimicked. This pH pattern represents the most physiological gastric pH conditions regarding healing soil ingestion. In the second alternative, the gastric pH value was set constant at 1.0, which represents the long-term limit of the measured gastric pH by the gastric pH-metry (Figure 2). Both stable gastric pH values (i.e. 2.0 and 1.0) include the mean and median value of 1.6 and 1.3 of the gastric pH-metry data, respectively.

The results of the solubility assays using different gastric pH statuses are shown in Figure 3. The indicated bioaccessibility values are based on the ^{238}U concentration of 2.59 mg/kg ± 0.08 mg/kg (mean ± SD) in the healing soil measured by ICP-MS after HF digestion. By using the stable gastric pH of 2, 7.6% ± 0.5% (mean ± SD) of healing soil-derived ^{238}U was soluble in the synthetic gastrointestinal fluid after centrifugation. Additional filtration steps (0.45 µm and 0.2 µm) did not markedly alter the bioaccessibility. Importantly, the bioaccessibility of ^{238}U did not change by adopting the alternative gastric pH status "physiological" from the gastric pH-metry, regardless of whether or not additional filtration steps were applied. A stable gastric pH value of 1 had no notable influence on the bioaccessibility of healing soil-derived uranium either. The bioaccessibility of the non-centrifuged but filtered samples also showed no obvious variations.

The results clearly demonstrate that there are no substantial changes of the estimated bioaccessibilities neither among the different gastric pH statuses used nor among the filtered or unfiltered samples. The bioaccessibility data obtained by using the stable gastric pH of 2, the

centrifugation and the filtration step at 0.2 µm was applied as the reference value. This resulted in a DF value of 7.7% ± 0.2% (mean ± SD).

Human Study – Determination of f_A

The ten volunteers who participated in the study ingested once a single amount of 20 g of healing soil which contained 51.8 µg ± 1.6 µg (mean ± SD) of ^{238}U. The daily urinary excretion of ^{238}U of the volunteers is shown in Figure 4. These data were further used to determine the volunteer-specific bioavailability f_A of healing soil-derived ^{238}U. The daily urinary excretion of ^{238}U over 3 days before healing soil-ingestion ranged from 11.4 ng/day to 12.7 ng/day (median values). This is in reasonable agreement with the results of a study on 113 German unexposed volunteers aged from 3 to 92 years, where a daily urinary excretion of ^{238}U of 14.4 ng/day (median) was measured.[21] Median excretion of ^{238}U after healing soil ingestion (day 4) was increased by about 6.4 ng ^{238}U compared to day 3 and was accompanied by an increased inter-individual variability. Over the next two days (day 5, day 6) the daily ^{238}U excretion declined towards blank values.

To determine the bioavailability (f_A) of healing soil-derived ^{238}U for each of the ten human volunteers, the current uranium biokinetic model published by the ICRP[12] together with the obtained urinary excretion data of ^{238}U were applied. A negative bioavailability of -0.01% was obtained for one subject due to a relatively high blank value of urinary ^{238}U at day 1, which presumably illustrates the strong intra-individual variation of dietary uranium excretion. This value was not included in further calculations. The results of the remaining nine volunteers are shown in Figure 5 (left graph). Similar to a former publication on daily urinary excretion of uranium[21], a log-normal distribution was assumed. Therefore, the f_A values are presented by a log-scaled probability plot in Figure 5 (right graph). Accordingly, geometric mean (GM) and

geometric standard deviation (GSD) were calculated to be 0.04% and 2.9, respectively. To cover a 95% confidence interval of f_A values, the 2.5th percentile and the 97.5th percentile were calculated by:[22, 23]

$$Q_{2.5th} = \frac{GM}{GSD^2} \quad (3)$$

and

$$Q_{97.5th} = GM \cdot GSD^2 \quad (4)$$

Thus, the 2.5th percentile and the 97.5th percentile amount to 0.0049% and 0.34%, respectively. The indicated range of f_A over three orders of magnitude implies a relatively high inter-individual variability.

The bioavailability (f_A) value of soil-derived uranium obtained in this experimental study is, to our knowledge, the first experimentally derived value in the literature. As mentioned, most of the former bioavailability (f_1) values obtained from human studies were from investigations on soluble uranium from drinking water. For this reason, these f_1 values are inappropriate for a direct comparison with f_A because of the relatively high proportion of insoluble uranium in soil. It is therefore more suitable to compare the f_1 values with the calculated f_A^{sol} values (see below). Similar to the dissolved uranium from drinking water, f_A^{sol} represents only the amount of soluble soil-derived uranium, which is absorbed from the alimentary tract into the circulatory system.

As already mentioned, to investigate any possible individual variations one male volunteer (Volunteer 1) participated three times. An interval of at least 2 months between the studies was maintained to avoid any interference. Thereby, the daily urinary excretion values of ^{238}U over 3 days before healing soil-ingestion were 3.9 µg/day ± 1.5 µg/day, 8.1 µg/day ± 0.6 µg/day and 9.0 µg/day ± 1.2 µg/day (mean ± SD). Similar variations are reported in the literature.[24] The corresponding f_A values were 0.024% ± 0.001%, 0.003% ± 0.001% and 0.044% ± 0.002% (f_A

295 value ± combined uncertainty). This limited data set suggests an intra-individual variation of the
296 f_A of about one magnitude, which was independent from the urinary ^{238}U status before healing
297 soil ingestion. This finding might indicate a general intra-variability of the uptake of uranium by
298 the alimentary tract.

299 Determination of f_A^{sol}

300 In the present study, the bioaccessibility and the corresponding bioavailability of healing soil-
301 derived ^{238}U were measured by a solubility assay and a human study. The bioaccessibility and
302 the bioavailability amounted to 7.7% ± 0.2% (mean ± SD) and 0.04% (GM) with a GSD of 2.9,
303 respectively. By adapting these results to eq. 1, f_A^{sol} results to 0.53% (GM) ranging from 0.06%
304 (2.5^{th} percentile) to 4.43% (97.5^{th} percentile).

305 The factor f_A^{sol} was established by using a healing soil, which contains only small amounts of
306 uranium. On the basis of a DF of 7.7%, the volunteers of the current study who ingested 20 g of
307 healing soil were only exposed to a small amount of soluble soil-derived uranium of about 4 µg.
308 However, the intention was to apply the f_A^{sol} to soil ingestion scenarios with high levels of
309 uranium. This raised the question as to whether f_A^{sol}, which was established by acute and low
310 levels of uranium, is also applicable to chronic and high level scenarios, or as to whether f_A^{sol}
311 might vary in these cases.

312 In this regard, the data on f_A^{sol} established in the present study was compared with the values
313 available in the literature on the absorption of high levels of soluble uranium in humans. As
314 already mentioned, f_A^{sol} and f_1 are suitable for comparison because both represent soluble
315 uranium, which is absorbed from the alimentary tract into the circulatory system. Table 1 gives
316 an overview on f_1 ranges for soluble uranium compounds obtained in different human studies
317 compared to the present value of f_A^{sol}. Three studies on acutely ingested uranium revealed f_1

values ranging from 0% to 5%. Three studies on chronic ingestion of soluble uranium showed similar data on f_1 ranging from 0.02% to 7%.

Table 1. Summary of bioavailabilities of ingested soluble uranium in humans

	Ingestion of soluble uranium	f_A^{sol} (present study) or f_1 for soluble uranium compounds (other studies)
Present Study	4 µg[a]	0.06% - 4.43%[c]
Karpas et. al. 1998[25]	100 µg[a]	0.14% - 1.56%
Harduin et. al. 1994[26]	162 µg[a]	0.5% - 5%[d]
Wrenn et. al. 1989[27]	~270 µg[a]	0% - 3.5%[d]
Harduin et. al. 1994[26]	81 µg/day[b]	0.3% - 2%[d]
Limson Zamora et. al. 2003[28]	0.37 to 573 µg/day[b]	0.1% - 6.3%
Karpas et. al. 2005[29]	10 - 2,775 µg/day[b]	0.02% - 7%

[a]Acute ingestion of uranium at one day. [b]Chronic ingestion of uranium, at least over 15 days. [c]Range for f_A^{sol} with a confidence interval of 95%. [d]Indicated ranges are based on interpretation of Leggett and Harrison et. al.[30]

Table 1 demonstrates that there are no major differences between the range of f_A^{sol} obtained in the present study and the ranges of the bioavailabilities (f_1) of former studies, no matter what levels, low or high, of uranium were acutely or chronically ingested. Based on the results in Table 1 it is assumed that the f_A^{sol} value does not depend on the amount or the exposure time of ingested uranium and can thus be used for soils highly contaminated with uranium. Additionally, similar to the human data, the bioavailability of soluble uranium e. g. in rodents ranged from 0.7% to 2.8%.[31, 32]

For the estimation of f_A and f_A^{sol}, only adult volunteers were considered. Hence, the derived data on f_A^{sol} should not be directly applied to children, since the latter might exhibit increased absorption and net retention of uranium, especially during periods of growth.[12, 15, 30, 33, 34] In this

regard, the derived value of f_A^{sol} is very likely to underestimate the actual bioavailability of non-adults.

Based on the f_A^{sol} value, it is possible to estimate more realistic f_A values for other soils as well. Assuming that the absorption from the alimentary tract does not depend on the chemical speciation of soluble uranium, only the solubility assay DIN 19738 has to be performed for a specific soil to gain the soil specific DF value.[35] A more realistic f_A value could then be achieved by directly applying eq. 1, without the need of performing additional human studies.

The resulting bioavailability f_A can be used in the biokinetic compartmental model of uranium to improve the internal dose assessment after soil ingestion. Currently we are working on the assessment of the internal dose after ingestion of some highly uranium-contaminated soils by applying the introduced concept.

ASSOCIATED CONTENT

Supporting Information

Section S.1: details on the determination of urinary ^{238}U by ICP-MS. Section S.2: details on the determination of ^{238}U from the solubility assay and the microwave-assisted digested healing soil by ICP-MS. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The authors would like to thank all volunteers for their participation. Thanks also to Prof. Dr. H. Feußner and H. Wirnhier (Department of Surgery, Klinikum rechts der Isar, Technische Universität München, Munich, Germany) for enabling us to conduct a gastric pH-metry. The present work was mainly funded by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF), Berlin, Germany (Contract No. 02NUK015B).

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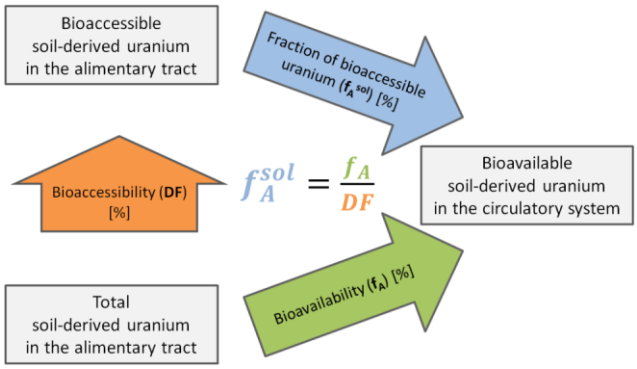
452 TOC/Abstract Art



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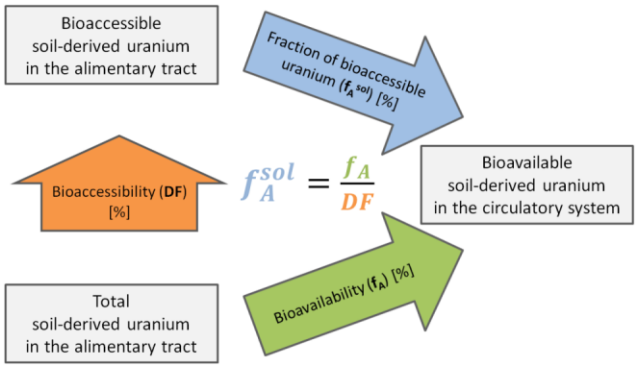

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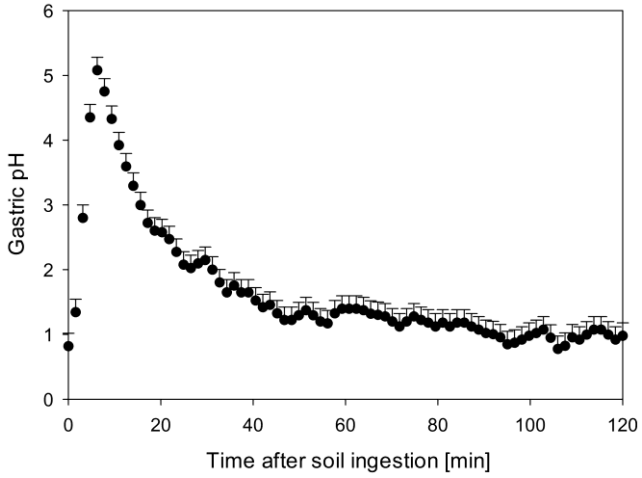
455 Figure 1. Scheme of the relation of bioavailability (f_A), bioaccessibility (DF) and the f_A^{sol} factor.



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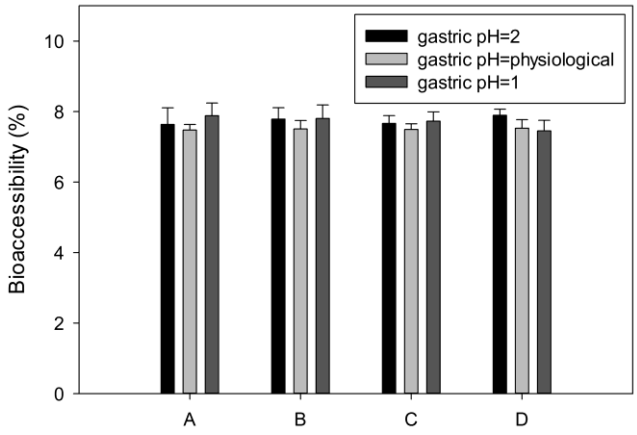
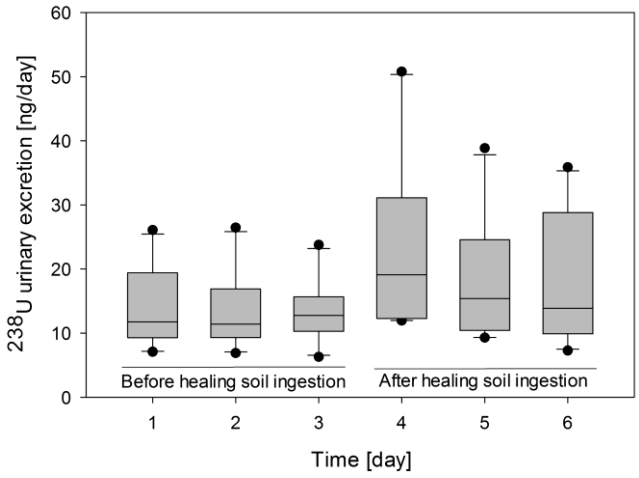
457 Figure 2. Gastric pH over 2 hours after a single initial ingestion of healing soil (20 g) by a male

458 volunteer (mean + standard uncertainty).

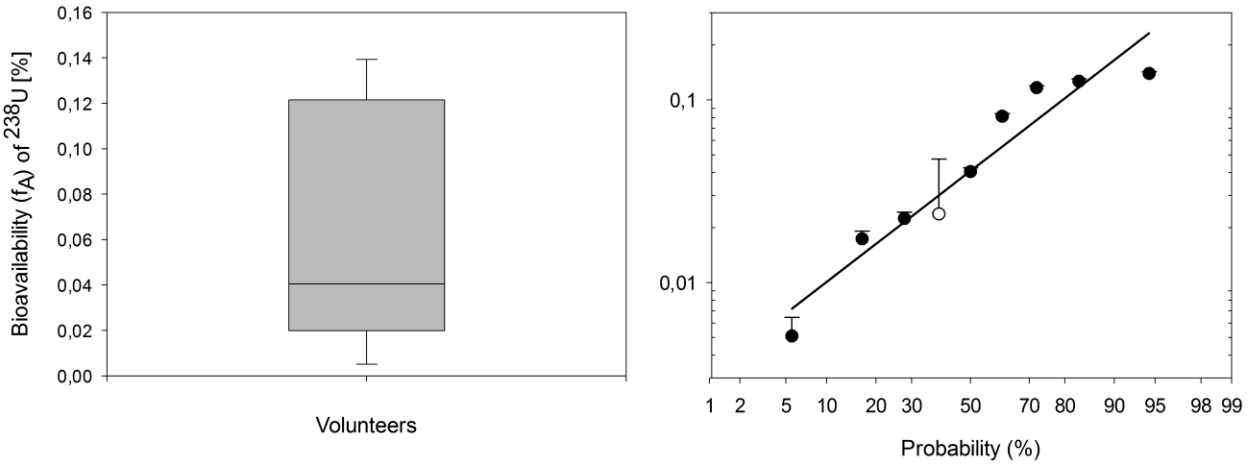
459 

460 Figure 3. Bioaccessibility (DF) of healing soil-derived ^{238}U in gastrointestinal fluid (mean + SD,
461 n=3). Before measurement via ICP-MS, the samples were (A) centrifuged, (B) centrifuged and
462 filtered at 0.45 μm, (C) centrifuged and filtered at 0.2 μm or (D) non-centrifuged, but filtered at
463 0.2 μm.



464

465 Figure 4. Daily urinary excretion of ^{238}U by ten volunteers. At the beginning of day 4, all
466 volunteers ingested 20 g of healing soil, equal to 51.8 µg of ^{238}U. Box plot with following
467 statistical values: 5^{th} percentile (lower circle), 10^{th} percentile (lower whiskers), 25^{th} percentile
468 (lower boundary of the box), median (solid line within the box), 75^{th} (upper boundary of the
469 box), 90^{th} (upper whiskers) and 95^{th} percentile (upper circle).



470

471 Figure 5. Bioavailability f_A of ^{238}U as experimentally derived on human volunteers ingesting
472 healing soil (n=9). Left graph: box plot with following statistical values: 10th percentile (lower
473 whisker), 25th percentile (lower boundary of the box), median (solid line within the box), 75th
474 (upper boundary of the box), 90th (upper whisker). Right graph: probability plot, including
475 experimental data with combined standard uncertainty (black dots with whiskers), Volunteer 1
476 with SD (open dot with whisker) and fitted curve (black line).

SUPPORTING INFORMATION

Estimating the absorption of soil-derived uranium in humans

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Section S.1:

For the analysis of ^{238}U an Element 1 ICP-SF-MS instrument (Thermo, Bremen, Germany) in low resolution mode was used. The samples of the solubility assay were diluted 1:2 with diluted nitric acid (5%, final concentration). The sample of the microwave-assisted digested healing soil was diluted 1:2 with diluted nitric acid (1%, final concentration). An internal standard solution (1 µg/L ^{193}Ir, final concentration) was added to each sample to correct for matrix interferences. For each sample three replicates were measured. Sample transport to nebulizer was realized by a peristaltic pump at a flow rate of 0.5 mL/min. Sample introduction to ICP-MS was performed by a Meinhard nebulizer fitting into a cyclone spray chamber. Uranium was determined at m/z = 238. RF power was 1200 W, nebulizer gas (Ar) was daily optimized and usually set to 0.8 L/min. Plasma gas: Ar, 15 L/min. Auxiliary gas: 0.8 L/min. runs: 3 patterns: 3, 12 samples per peak. The instrument was calibrated using a 7 point calibration between blank and 2000 ng/L. After ten measurements regularly three blank determinations and a control determination of a certified standard were performed. Calculation of results was carried out on a computerized lab-data management system, relating the sample measurements to calibration curves, blank determinations and control standards. The detection limit, calculated as blank + 3 times the blank standard deviation (SD) was 1.5 ng/L, the limit of quantification (LOQ) calculated as blank + 10 x SD was 4.5 ng/L.

Section S.2:

The determination of urinary ^{238}U was carried out according to the DIN EN ISO 17294-2 by inductively coupled plasma mass spectrometry on an Element 2 (Thermo Scientific).[1] For the determination of the ^{238}U concentration in urine, the calibration and data evaluation were carried out by applying the standard addition method. Thus, the elimination of disturbing influences was assured as far as possible by the usage of the same matrix as the real sample. For the standard addition six aliquots of one urine sample were diluted with 1.5% nitric acid in a 1:10 ratio. In five dilutions, uranium was added to yield concentrations from 0.005-0.025 µg/L. The resulting calibration curve was checked with the reference material SeroNorm Trace Elements Urine. Subsequently, the real samples were diluted with 1.5% nitric acid in a 1:10 ratio and measured several times. ^{103}Rh was used as the internal standard.

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