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Methods/Techniques:	Toxicology - pharmacokinetics, Metals - bioavailability, Toxicology - animal study
Additives/Contaminants:	Heavy metals - cadmium

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Food Types:	Animal products – meat
Abstract:	<p>Growing male pigs were exposed to cadmium (Cd) at levels around 1 and 10 mg/kg feed for up to 12 weeks, administered as CdCl₂ or Cd-cysteine (CdCys). Pigs exposed to 10 mg/kg showed decreased growth during the last 3 weeks.</p> <p>Liver and kidney concentrations of Cd continuously increased over the entire 12 week exposure, exceeding the EU limits of 1 (kidneys) and 0.5 mg/kg (liver) within 3 weeks at the feed level of 10 mg/kg. A switch to clean feed after 3 weeks for 5 or 9 weeks, resulted in steadily decreased levels in kidney and liver, which could be completely attributed to organ growth. At the lower feed level, the level in kidneys exceeded the limit almost 2-fold after 12 weeks, but not after 3 weeks. Liver levels remained below the limit.</p> <p>Metallothionein (MT) levels in livers showed a steady decrease in both untreated and treated animals over time. In kidneys such a decrease was only observed in control animals whereas in the highest dosed animals the MT concentrations steadily increased.</p> <p>The observed carry-over of Cd from feed to liver and kidney was modelled by means of a simple transfer model relating levels in feed via MT levels to accumulation of Cd. Using this model, it was shown that the exposure period of growing pigs to feed containing the EU limit of 0.5 mg/kg feed should be less than 12 weeks in order to prevent Cd levels in the kidneys to exceed the EU limit.</p>

Abstract

Growing male pigs were exposed to cadmium (Cd) at levels around 1 and 10 mg/kg feed for up to 12 weeks, administered as CdCl₂ or Cd-cysteine (CdCys). Pigs exposed to 10 mg/kg showed decreased growth during the last 3 weeks.

Liver and kidney concentrations of Cd continuously increased over the entire 12 week exposure, exceeding the EU limits of 1 (kidneys) and 0.5 mg/kg (liver) within 3 weeks at the feed level of 10 mg/kg. A switch to clean feed after 3 weeks for 5 or 9 weeks, resulted in steadily decreased levels in kidney and liver, which could be completely attributed to organ growth. At the lower feed level, the level in kidneys exceeded the limit almost 2-fold after 12 weeks, but not after 3 weeks. Liver levels remained below the limit.

Metallothionein (MT) levels in livers showed a steady decrease in both untreated and treated animals over time. In kidneys such a decrease was only observed in control animals whereas in the highest dosed animals the MT concentrations steadily increased.

The observed carry-over of Cd from feed to liver and kidney was modelled by means of a simple transfer model relating levels in feed via MT levels to accumulation of Cd. Using this model, it was shown that the exposure period of growing pigs to feed containing the EU limit of 0.5 mg/kg feed should be less than 12 weeks in order to prevent Cd levels in the kidneys to exceed the EU limit.

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25 **Introduction**

26 Heavy metals like lead, mercury and cadmium may present a human health risk following the
27 consumption of animal derived products which are contaminated due to the carry-over from
28 contaminated feed. In the case of Cd, the toxic risk is aggravated by its accumulation in the
29 target organs kidney and liver. Cd may occur as a contaminant in minerals used as feed
30 ingredients (Sapunar et al. 2001), but also be present in plants grown on soil of contaminated
31 areas, like in the Kempen in Belgium and the Netherlands due to the production of zinc (Franz
32 et al. 2008). Also the application of contaminated sewage (Lisk et al. 1982, Bache et al. 1987)
33 or manure (Lindén et al. 1999) may lead to elevated levels. In addition, the issue may be
34 relevant due to intake of contaminated soil by animals. To ensure the safety of consumers,
35 maximum limits have been set by the EU for both food and feed with e.g. limits for pig feed
36 being 0.5 mg/kg (EU 2002) and, 1.0, 0.5 and 0.05 mg/kg for pig kidneys, livers and meat
37 respectively (EU 2006).

38 In order to guarantee that limits for feed are low enough to ensure compliance of various
39 products of food producing animals, it is important to understand the relationship between
40 levels in feed and food. As shown previously for dioxins and PCBs, and aflatoxin B1
41 (Eijkeren et al. 2006a,b, Hoogenboom et al. 2007, 2010), such knowledge may not only help
42 in tuning feed to food limits, but also be important for evaluating the potential risks during an
43 incident and for calculating the duration of the period on clean feed to return to compliant
44 levels in food. Aim of the present study was to study the transfer of Cd in growing pigs and to
45 model this transfer to the organs of interest, i.e. liver and kidneys. Though various studies
46 have been performed with Cd in growing pigs (Bache et al. 1987, King et al. 1992, Zacharias
47 et al. 2001), none of these studies included different time points of exposure and the effect of
48 changing the contaminated feed by clean feed, information required for proper modelling of
49 the data. To overcome this deficiency, a study on the kinetics of Cd was performed, including
50 the levels in various organs and blood. As metallothioneins (MTs) play an essential role in the
51 accumulation of Cd in tissues like liver and kidney (Margoshes et al. 1957, Kägi and Vallee
52 1960, Henry et al. 1994), levels of these proteins were determined in these organs. The
53 experimental data were used to develop a mechanistically based transfer model for Cd to
54 these organs.

55 Another important fact is the speciation of Cd in feed. For example, when plants are grown
56 on contaminated soil, part of the Cd may be converted to organic forms which upon ingestion
57 by animals may show different kinetics than the inorganic form. For this reason, Cd-cysteine
58 was prepared and next to CdCl₂ used to expose the pigs.

Materials and Methods

Materials

Cadmium chloride (CdCl_2) was purchased from Merck (Darmstadt, Germany). Cadmium-cysteine (CdCys) was prepared as described by Cherian (1979) by mixing a solution of cysteine and CdCl_2 and adjusting the pH to 8 with NaOH. At this pH, the CdCys precipitated and was collected by centrifugation. To increase the yield, CdCl_2 was added to the supernatant until the remaining cysteine precipitated as well. The CdCys was lyophilized and the Cd content determined with Electrothermal Atomic Absorption Spectrometry (ETAAS).

Feeds were prepared by Research Diet Services (Wijk bij Duurstede, The Netherlands), both starter and grower feeds, without or with either CdCl_2 or CdCys. Feed composition, in g/kg, was as follows: barley 347.8, wheat 200.0, peas 50.0, soybean meal 150.0, wheat semolina 60.0, rapeseed meal 50.0, sugarcane molasses 20.0, potato protein 25.0, whey powder 40.0, soy oil 34.5, chalk 13.3, premix ID for growth 2.0, dicalcium phosphate 4.5, NaCl 2.5, lysine HCl 0.2 and DL methionine 0.2. Cd was added as CdCl_2 or CdCys and Cd levels were aimed at 1 and 10 mg per kg. Levels were checked by analysis (see Results).

Animal study

The animal study was carried out at ASG in Lelystad. The study was reviewed and approved by the Ethical Committee. Three-month old male pigs were divided in various treatment groups, as shown in Figure 1:

- Group 1 was the control group and received no additional Cd. Before starting the treatment, 3 animals were slaughtered (time 0) and subsequently at 3 and 12 weeks (both 3 animals). After 3 weeks the starter feed was replaced by grower feed.
- Groups 2 and 3, each consisting of 12 animals, received feed with respectively 1 or 10 mg Cd, as CdCl_2 , per kg feed for a period of 3 weeks. At that time-point 3 animals per group were slaughtered. Another 3 pigs of each group were switched to grower feeds with the same levels of CdCl_2 for another 9 weeks. The remaining 6 animals were put on clean grower feed in order to mimic a short-term incident with contaminated feed. From these 6 animals in each group, 3 were slaughtered after 5 weeks and 3 after 9 weeks.
- Two additional groups (4 and 5) of each 12 animals received CdCys at an intended Cd level of 1 and 10 mg/kg feed for 3 weeks and were subsequently switched to grower feeds with similar contamination for another 9 weeks or put on clean grower feed for 5 or 9 weeks.

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93 Animals were housed in groups of 3. Feed was supplied ad libitum. During the first 3
94 weeks the starter feed was supplied on specific days, starting with 44 kg on day 1 and adding
95 20 kg on days 7, 13, 17 and a varying amount on day 18 depending on the consumption thus
96 far. On day 22 the remains were weighed to determine the overall feed consumption per cage
97 of 3 animals. At day 22 a portion of 44 kg grower feed was supplied followed by 20 kg every
98 3 days. On day 57 the remains were weighed and the overall consumption determined for the
99 period 3-8 weeks. On day 57, per cage 50 kg was supplied followed by 20 kg every second or
100 third day. Remains were determined on day 84 to calculate the feed consumption over the
101 period of 8-12 weeks. Animals were weighed at day 0 and after 3, 8 and 12 weeks, when part
102 of the animals were slaughtered.

103 Blood samples were collected from the animals kept on contaminated feed for 12 weeks on
104 days 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 and 84. Animals transferred to clean feed after 3
105 weeks were sampled on days 1, 3, 7, 14, 21, 22, 24, 28, 35, 42, 49, 56, 63, 70 and 84.

106 Animals were slaughtered after 0, 3, 8 and 12 weeks. Various tissues were sampled,
107 including liver, kidney, muscle, duodenum, ileum, jejunum, lung, brains, testicles and bones.

108

109 *Analysis of cadmium*

110 Samples were pre-treated using acid digestion with a microwave oven (MARS 5). In short, 1
111 gram of feed or 2 grams of liver, kidney, muscle, other tissue or blood was mixed with 10 ml
112 70% HNO₃ and heated in a microwave oven to a temperature of 210°C.

113 Acid digests were analyzed using an Electrothermal Atomic Absorption
114 Spectrophotometer (ETAAS) with Zeeman background correction, using a graphite coated
115 furnace and graphite tube to vaporize the sample. Cd concentrations were determined from a
116 working curve (linear) after calibrating the instrument with standards of known concentration.
117 The Cd concentrations were measured at 228.8 nm.

118 The limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be
119 quantitatively determined with suitable precision and accuracy. For this study the LOQ was
120 10 µg/kg for the liver, kidney, muscle and other tissues. For the blood samples the LOQ was 1
121 µg/kg.

122

123 *Analysis of metallothioneins*

124 For determination of metallothioneins (MT), kidneys and livers were partially thawed
125 overnight in a cooled chamber and homogenised as a whole in a commercial food blender. A
126 subsample of approximately 1 g was subsequently homogenised on ice with 4 volumes of

buffer A (10 mM tris-HCl, 86 mM NaCl, pH 7.4) at 4°C with an Ultra-turrax T3 homonizer (IKA, Labor technique, Shaufer, Germany). Tissue and cell homogenates were centrifuged at 16,000g at 4°C in 20 minutes and supernatant fractions were taken and kept at -80°C for further processing.

Cytosols of the tissues were analysed following the procedure described by Klein et al. (1994). This method allows the quantification of total MT in biological materials, including the oxidised and aggregated MT, since particularly Cu-containing MT has the tendency to polymerise. This was a modification of an earlier described procedure specially developed to quantify Cu-containing MT (Klein et al. 1990). The main features of the procedure are that oxidised MT is converted into native MT with 2-mercaptoethanol as a reducing agent and Zn^{2+} as a metal donor, and MT is subsequently quantified via Cd saturation. High molecular weight Cd-binding compounds are denatured with acetonitrile, Cu bound to MT is removed with ammonium tetrathiomolybdate and excessive tetrathiomolybdate and its Cu complexes are removed with DEAE-Sephacel (Sigma, St. Louis, MO). Subsequently apothionein is saturated with ^{109}Cd -labeled $CdCl_2$ -solution (Amersham Pharmacia Biotech, Buckinghamshire, England: 50 ppm of 37 MBq/mg Cd in 0.1 M HCl), and excessive Cd is bound to Chelex 100 (Bio-Rad, Munich, Germany). The precipitate was removed by centrifugation and the supernatant counted for 1 min in a gamma counter (Minaxi γ , Canberra Packard, Frankfurt, Germany). The MT concentration was calculated assuming a molar ratio of Cd/MT of 7.

Modelling of the data

The modelling of the transfer of contaminants from feed to organs of growing animals requires detailed information on organ growth as well as its concomitant Cd accumulation. As such information was available for liver and kidneys, a mechanistic transfer model could be developed for these organs, i.e. a model relating levels in feed to organ levels due to binding of Cd to metallothioneins. However, in the case of the blood the absence of growth data of this compartment only allowed a strict empirical modelling approach, i.e. a description of the time course of the accumulation and depletion kinetics of Cd in the blood.

Red blood cells

Analysis of the plasma samples showed concentrations systematically below the detection limit of 1 $\mu g/kg$, but analysis of the red blood cell (RBC) samples clearly showed an increase of Cd during the exposure phase and decrease during the phase on clean feed. Therefore, the

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modelling in blood focussed on the data in RBCs. An one-compartment classical kinetic model for Cd in RBCs was assumed to be:

$$C(t) = \begin{cases} C_0 + \Delta C_{ss} \cdot (1 - \exp(-k \cdot t)) & t \leq T \\ C_0 + \Delta C_{ss} \cdot (1 - \exp(-k \cdot T)) \cdot \exp(-k \cdot (t - T)) & T < t \end{cases} \quad (1)$$

The motivation of such a model is that due to continuous background contamination the concentration of Cd in RBCs at the start of the experimental period will have led to a constant background concentration, i.e. C_0 . After start of the experiment, the concentration increases during the exposure period of duration T towards an assumed steady state elevation level ΔC_{ss} due to uptake of additional Cd ingested through contaminated feed. Furthermore, two processes are involved that tend to decrease the concentration with an exponential rate k : dilution due to animal (and thus RBC-tissue) growth and elimination due to turn-over of RBCs. Due to these processes the Cd concentration will decrease once the exposure to contaminated feed has stopped. .

Liver and kidney

In concordance with earlier findings in livestock (Beresford et al. 1999; EFSA, 2004; Crout et al. 2004; Eijkeren et al. 2007), the accumulation of Cd in organs like the liver and the kidneys kinetically may be described as an *irreversible* uptake process. The transfer model for Cd in feed to the liver and the kidneys therefore describes the accumulation of Cd in these organs as irreversible MT binding depending on the dietary amount of Cd taken in (A_{feed}), or:

$$\frac{dA}{dt} = \frac{\alpha A_{feed}}{\beta + A_{feed}} \cdot MT \quad (2a)$$

with:

- dA/dt organ specific Cd uptake rate (mg/day)
- MT organ specific metallothionein concentration ($\mu\text{mol/kg}$)
- A_{feed} daily Cd intake (mg/day)
- β parameter characterising saturable transfer of Cd from feed to MTs (mg/day)
- α parameter scaling the organ MT level to the Cd uptake rate ((kg/ μmol). (mg/day))

Note that the parameter β has to be interpreted as characterising the overall saturable binding involved in the transport of Cd from feed to organ MTs. Next to the MT binding this may be the (yet unidentified) binding of Cd to transport proteins in the blood, the GI tract, etc.

Furthermore note that equation 2a as such prescribes the continuous accumulation of Cd in the kidneys and the liver as long as the exposure continuous. Once the exposure is stopped the absolute amount of Cd in these organs basically remains constant, unless decreasing due to organ growth in the period after the exposure.

Integrating equation 2a yields the absolute amount (A , mg) of Cd in the liver and the kidney, which may be converted to the organ Cd concentration (C , mg/kg wet tissue) by division through organ weight (W), or:

$$C=A/W \quad (2b)$$

Being suited for growing pigs the model includes a linear model for organ growth, or:

$$W=W_0+dW.t \quad (2c)$$

with W_0 being the organ weight (kg) at the start of the exposure period, dW the organ growth rate (kg/day) and t the duration of the exposure in days. Organ weight (W) is assumed to increase linearly in time during the experimental period.

With regard to the MT concentration, Verma et al. (1978) found a linear relationship for organ MT with organ Cd concentration for a wide range of Cd concentrations in kidney and liver of cows, chickens and swine with correlations of 0.90 and 0.43 in swine kidney and liver, respectively. So, for the MT concentration a linear model was assumed, or:

$$MT=MT_0+dMT.C \quad (2d)$$

with MT_0 as the metallothionein concentration ($\mu\text{mol/kg}$ wet weight) at the start of the exposure period and level before the treatment and dMT ($\mu\text{mol/kg}$ wet weight /day) as the rate at which the metallothionein level changes as function of the organ Cd concentration during the treatment period (regression parameter).

The carry-over rate (COR) is defined as the ratio between the organ-specific uptake rate (dA_{organ}/dt) and the daily Cd intake (dI/dt), or:

$$COR = \frac{dA_{organ} / dt}{dI / dt} \quad (3a)$$

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223 Equation 2a can be interpreted in terms of the COR as:

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$$COR = \frac{\alpha}{\beta + A_{feed}} \cdot MT \quad (3b)$$

225 Note that in the present study the COR is not constant but depends on A_{feed} and MT .

226

227 *Statistical analysis*

228 Observed differences were tested for significance using the Students t-test and based on a
229 two-sided comparison.

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Results and Discussion

Cadmium levels in feed

In principle, five different types of feed were prepared, a blank feed, two feeds with CdCl₂ and two with Cd-cysteine (CdCys), aiming for both types of Cd at levels of 1 and 10 mg Cd/kg. Regarding the age of the animals, both starter and grower feeds were prepared of each type of feed. Levels in the starter feed were determined to be 0.06 mg/kg in the blank feed, 0.78 and 9.5 mg/kg in the feeds with CdCl₂, and 1.00 and 9.4 mg/kg in the feeds with CdCys. Levels in the grower feeds were determined to be 0.88 and 10.0 mg/kg in the feeds with CdCl₂, and 0.84 and 10.0 mg/kg in the feeds with CdCys. Levels are expressed on product base but moisture content varied between 11.2 and 11.6%, i.e. very close to the 12% which is the basis for the EU limit of 0.5 mg/kg. So in principle, feed levels were around the intended levels.

Feed consumption

The animals received the feed ad libitum but the amount consumed per cage of 3 animals was determined after 3, 8 and 12 weeks and converted to an average feed consumption per animal (Table S1). Due to the social housing type, it was not possible to determine the feed consumption for each individual animal. Feed consumption in the control animals kept for the whole 12 weeks increased from 1.27 kg/animal/day during the first 3 weeks to 2.03 in weeks 3-8 and 2.70 in the final 4 weeks.

During the first period of 3 weeks there was no clear treatment related effect on feed consumption. In this period a larger number of animals received the same treatment with average consumptions for the control of 1.34 kg/pig/day (2 cages, 6 pigs), 1.38 and 1.45 kg/pig/day for the 1 and 10 mg/kg CdCl₂ groups (4 cages, 12 pigs each treatment) and 1.36 and 1.36 kg/pig/day for the 1 and 10 mg/kg CdCys groups (4 cages, 12 pigs each treatment).

During the second period (3-8 wks) there also seemed to be no consistent differences between the groups. However, in the third period (8-12 wks), the feed consumption in the two groups exposed to 10 mg/kg for the whole 12-weeks period (coded 12/0) seemed clearly lower than that of either the control pigs, the pigs on the lower dose or the pigs that switched to clean feed after 3 weeks. However, there was quite some variation in the data and it is difficult to draw firm conclusions.

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Based on feed levels and intake, the total intake of Cd per pig during the 3, 8 or 12 weeks of the study were calculated (Table S1), with the largest intake for the two groups fed on 10 mg/kg CdCl₂ or CdCys for 12 weeks, both being around 1.5 g/pig.

Body and organ weights

Table S2 shows the body weight of the pigs at the time of slaughter, as well as the absolute and relative weights of livers and kidneys. Control pigs grew from about 20 kg before the start of the treatment to respectively 38 and 97 kg after 3 and 12 weeks. Pigs were also regularly weighed during the study, allowing monitoring of the growth of individual animals for the whole study period, i.e. those not being slaughtered at intermediate times. Figure 2 shows the progression of the body weights of the untreated pigs and the pigs exposed to the high and low dose of CdCl₂ or CdCys for 12 weeks, or 3 weeks followed by a 9 weeks period on clean feed. Regarding the absence of clear differences in body weight, the data for CdCl₂ or CdCys were combined. The pigs exposed to the dose of 10 mg/kg feed for 12 weeks, showed less growth during the last 5 weeks. This difference was not significant (p=0.17) when compared to the 3 control pigs but highly significant (p= 0.00004) when compared to the combined controls and low dose (1 mg/kg) groups. This was not the case for the pigs from the high dose group that switched after 3 weeks to clean feed (p=0.66).

In the case of 10 mg/kg CdCl₂, weight increase in this last period was 19.9 ± 3.4 kg, as compared to 29.5 ± 2.2 kg in the controls and 29.5 ± 2.2 and 27.3 ± 3.1 kg in the animals exposed to 1 mg/kg feed for 3 (+ 9 weeks clean feed) or 12 weeks respectively. The animals exposed to the feed with 10 mg/kg for 3 weeks followed by 9 weeks on clean feed, showed an increase of 22.9 ± 2.5 kg, again less than the control and low dose (1 mg/kg) groups. In the case of CdCys, the pigs exposed to the high dose also showed a decreased growth during the last 3 weeks, being 18.2 ± 2.8 kg, but in this case the group exposed for only 3 weeks followed by 9 weeks on clean feed showed a normal growth of 26.0 ± 9.1 kg and the animals exposed to 1 mg/kg for 3 and 12 weeks showed a growth of respectively 24.4 ± 1.7 and 28.7 ± 1.2 kg. This decreased growth was accompanied by a reduced feed intake but also an increased feed conversion ratio (Table S1). An increased feed conversion ratio was also observed in the last period for the group exposed to 10 mg/kg CdCl₂ for 3 weeks followed by clean feed for 9 weeks, being the group that also showed a decreased growth.

The liver and kidney weights reflected the body weights, showing a reduction in the case of the animals exposed to the high dose of CdCl₂ or CdCys for the whole period (Table S2).

Overall these data indicate that prolonged feeding with feed containing 10 mg/kg Cd causes some kind of adversely changed growth physiology in the animals, leading to a reduced growth. Alternatively, pigs are more susceptible to Cd during this last period of 4 weeks. Phillips et al. (2003) treated pigs for 137 days and also observed a decreased growth and increased feed conversion ratio at a dose of 2.5 mg/kg feed. Similar was true for Xu et al. (2004) who exposed pigs for 12 weeks to 10 mg/kg Cd in the feed for 12 weeks and observed a slight decrease in feed intake but a clearly decreased growth and increased feed conversion ratio. However, King et al. (1992) fed pigs a wheat-based feed contaminated with CdCl₂ for their whole lifetime (8-90 kg) with Cd levels up to 4.4 mg/kg but did not observe any effect on feed intake or growth rate. Tu et al. (2007) treated pigs for 12 weeks and observed an increased relative liver weight at a dose level of 10 mg/kg, as well as an increase in serum glutamic-pyruvic transaminase (GPT) and decreased Na⁺/K⁺-ATP-ase activity in liver, both indicative of liver damage. Unfortunately, body weights and feed consumption were not reported. In the current study no effect on relative liver weight was observed (Table S2). Nevertheless, these studies indicate that the higher dose levels may to some extent affect pigs and that the current EU feed limit of 0.5 mg/kg also protects pigs for these potential adverse effects of Cd.

For the transfer model (equation 2) for the Cd concentration in liver and kidney, an organ weight model was required. Figure S1 shows the liver and kidney weights at 3, 8 and 12 weeks after the start of the experiment. When combining all animals, the linear models describing the data are: liver weight (gram) = 626 + 100 × time (weeks); kidney weight (gram) = 114 + 26 × time (weeks).

Metallothioneins in kidneys and liver

Exposure to heavy metals is known to trigger the formation of metallothioneins (MT) which subsequently bind the metals. Since this phenomenon is important for modelling the data, MT levels were determined in livers and kidneys of all animals. Again, there were no clear differences between the CdCl₂ or CdCys groups and therefore data were combined. As shown in Figure 3 (upper left), MT levels in kidneys of control animals decreased with age from an initial 12.5 ± 0.4 µmol/kg at the start of the experiment to 10.0 ± 3.4 µmol/kg and 6.2 ± 1.0 µmol/kg after an additional 3 and 12 weeks. Exposure to the high Cd dose (10 mg/kg) resulted in increased levels of 14.8 ± 2.1 and 20.4 ± 0.5 µmol/kg after 3 and 12 weeks. When the exposure was stopped after 3 weeks, levels decreased to 13.0 ± 1.7 and 10.5 ± 1.2 µmol/kg at 8 and 12 weeks, the latter still being almost double the level in the control group.

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This is in line with the increased Cd levels in these kidneys (see below). In the animals exposed for 12 weeks to the low dose (1 mg/kg), the MT levels at 3 and 12 weeks were respectively 10.3 ± 1.7 and 9.2 ± 1.4 $\mu\text{mol/kg}$, meaning no difference at 3 weeks but not showing the further decrease observed in the controls at 12 weeks. When exposed to this low dose for only 3 weeks, followed by clean feed, there was no difference with the control animals at 12 weeks.

In the case of the livers (Figure 3, upper right), initial MT-levels at the start of the study were 5-fold higher than in kidneys, being 60.0 ± 7.4 $\mu\text{mol/kg}$. Levels were similar to those reported for pig livers by Henry et al. (1994). MT levels decreased to 22.9 ± 3.0 and 19.5 ± 3.8 $\mu\text{mol/kg}$ after 3 and 12 weeks in the control animals. In the animals exposed to the highest Cd levels for 3 and 12 weeks, levels were respectively 23.0 ± 3.6 and 23.4 ± 4.3 for CdCl_2 , and 16.5 ± 2.5 and 20.7 ± 3.8 $\mu\text{mol/kg}$ for CdCys. So contrary to the kidneys, there was no clear effect of the high Cd exposure on the MT levels in livers. López-Alonso et al. (2012) investigated cadmium and MT levels in pigs raised under intensive and extensive production systems. MT levels in livers were similar between the two groups, but in kidneys the MT levels were 50% higher in the intensively reared pigs, which was attributed to the difference in supplementation with zinc and copper. Cadmium levels in both livers and kidneys were much higher in intensively reared pigs. In general, MT levels in livers were much higher than in kidneys.

In order to model the Cd levels in liver and kidney, a model for Cd induced MT levels in these organs is required (equation 2). Figure 3 (lower panels) shows the relation between Cd and MT levels in kidneys (left) and livers (right). In kidneys, regression was applied of MT levels in dependence of Cd levels following the linear MT induction model of equation (2), resulting in values for $MT_0 = 8.65$ (SD: 0.37) and $dMT = 0.923$ (SD: 0.081). Based on this model, for the lowest dose applied, the MT levels were estimated to be 9.1 $\mu\text{mol/kg}$ after 3 weeks and 9.0 and 10.0 $\mu\text{mol/kg}$ after 12 weeks for halted (3+9) and continued exposure (12+0), respectively. For the highest dose applied, MT levels were 13.1 $\mu\text{mol/kg}$ after 3 weeks and 10.6 and 21.0 $\mu\text{mol/kg}$ after 12 weeks for halted and continued contamination, respectively. In liver, the regression coefficient - 0.9 (SD 1.8) for the estimated MT levels in dependence of Cd levels appeared to be insignificant. Mean MT-level in the liver is 22.3 $\mu\text{mol/kg}$ with a standard deviation of 12.2, reflecting the large variability in MT levels detected.

Cadmium levels in liver and kidneys

Table 1 shows the levels of Cd in the livers and kidneys of the animals, both as concentration and as absolute amounts based on the organ weights, Figure S2 shows the combined data for pigs treated with CdCl₂ and CdCys. It is evident that at both doses in the feed there is a time related increase in the levels in both tissues with on average a four- to five-fold higher level in the kidneys. Such differences were also reported by other groups and shows that kidney is the major organ in terms of concentrations (Bache et al. 1987, King et al. 1992, Zacharias et al. 2001, see Table S5). The levels after 12 weeks on the contaminated feeds were roughly three times higher than at 3 weeks. The tenfold difference in the levels of the low and high dose is also reflected in the levels in both kidneys and livers at 3 and 12 weeks, possibly with the exception of the high CdCys group at 12 weeks. Levels observed by King et al. (1992) in animals fed a CdCl₂ fortified diet were similar for livers but up to 2 times higher in kidneys. Zacharias et al. (2001), also supplying CdCl₂ but slaughtering the animals at 30 and 50 kg, observed much higher levels in both liver and kidneys. These authors demonstrated that other components in the feed like phytase and calcium might have an influence on the bioaccessibility and hence absorption of Cd in the GI-tract. At the same time, in the present study, kidney levels in the control animals after 12 weeks (0.08 mg/kg) were much lower than observed by previous authors, being 0.78 (Bache et al. 1987), 0.39 (King et al. 1992), and 0.6 mg/kg (Zacharias et al. 2001) despite the similar levels in the control feed, being around 0.06 mg/kg. This suggests that other sources than the feed could also have contributed to the exposure in some of these studies or that the animals already contained higher levels at the start of the study.

During the exposure period, the weights of livers and kidneys increased, both by a factor of 2 to 2.5 between 3 and 12 weeks. Table 1 also shows the total amount of Cd in the livers and kidneys, demonstrating that livers and kidneys contain similar absolute amounts. Between weeks 3 and 12, there is a 6 to 8-fold increase in the absolute amounts in the livers and kidneys in the low dosage groups, but in the high dosage groups this relative increase is less, being 5 to 6-fold. This may be related to the differences in the lower increase in liver and kidney weights in the higher dosage groups. When compared to the total intake of Cd, the relative amounts retained in liver and kidneys in the low dose groups were respectively 0.47 and 0.44%, and in the high dose groups 0.36 and 0.34%.

Following the switch at 3 weeks to clean feed, there was initially an increase in the absolute amounts in both kidneys and livers in some of the groups, like the low dose CdCl₂ group. There was hardly any change in the next 4 weeks, neither in kidneys nor in livers. The

suggestion that Cd binds to MT in the liver and is then transported to the kidneys, where it accumulates seems thus very unlikely.

The model in equation (2), given the organ growth models and the MT induction models described above, was applied for fitting the parameters α and β to the data on the amounts after 3 and 12 weeks continuous exposure for both liver and kidney. The fitted values for the model parameters are $\alpha = 7.2 \times 10^{-3}$ (SD: 0.7×10^{-3}) and $\beta = 12.4$ (SD: 2.0) for kidney and $\alpha = 8.4 \times 10^{-3}$ (SD: 1.3×10^{-3}) and $\beta = 237.0$ (SD: 7.4) for liver. Data on the absolute amounts of Cd in kidney and liver for the animals exposed for a continuous period of 3 or 12 weeks are compared in Figure 4, to the resulting model. Modelled data for the resulting concentrations in kidneys and livers at the two doses of CdCl₂ and CdCys are depicted in Figure 5, showing a good fit both during continuous exposure and after the switch to clean feed. The latter data may serve as a model verification. These graphs take into account the growth-related increase in the organ weights.

Studies in different animal species have shown a low absorption of ingested Cd (0.5 to 7%, EFSA, 2004). The results of the present study are in line with such low absorption and, hence, a low carry over to the animal. For example, given the model parameterisation (equation 2a) the daily uptake in the kidney amounts up to 0.1 mg/day in animals exposed for 12 week to feed containing 10 mg Cd/kg. Given a daily intake of 22 mg Cd this corresponds with a COR of 0.45%.

The calibrated model was used to simulate the accumulation of Cd in the kidneys and the liver for feed containing 0.5 mg/kg. The result of this simulation, which is shown in Figure 6, indicates that Cd levels in kidneys of pigs exposed to Cd at the EU limit in feed will exceed the EU limit after 12 weeks of exposure. In the case of the liver the EU limit in feed prevents that, even after a prolonged exposure period.

Cadmium levels in other tissues and hair

Levels in muscle tissue in all dosage groups were below the LOQ of 0.01 mg/kg, which is five times under the maximum limit for Cd in the EU (data not shown). This is in line with previous studies (Bache et al. 1987, Lisk et al. 1982, Sapunar-Postružnik et al. 2001, Zacharias et al. 2001). Similar was true for the brains, showing no detectable Cd levels in any of the groups.

There was a slight increase in the Cd levels in the testes of the high dosage groups after 3 and 12 weeks, being 0.020 ± 0.010 and 0.049 ± 0.007 mg/kg for the CdCl₂ group, and 0.023 ± 0.003 and 0.046 ± 0.026 mg/kg for the CdCys group as compared to non-detectable (<0.010)

in the control group (Table S1). The lungs in these dosage groups showed Cd levels of 0.083 ± 0.032 and 0.079 ± 0.002 at week 3 and 0.230 ± 0.040 and 0.167 ± 0.084 mg/kg at week 12. Levels decreased upon the switch to clean feed at 3 weeks but were still detectable after 9 weeks (Table S1). Philips et al. (2003) also examined Cd levels in testes and lungs of pigs exposed to doses up to 2.5 mg Cd/kg feed for 137 days, but observed levels below 0.003 mg/kg in these tissues, despite some dose-related increase.

As shown in Table S2, levels in the duodenum were clearly increased in the high dosage groups at 3 and 12 weeks, 4.30 ± 2.80 and 1.12 ± 0.17 mg/kg for the CdCl₂ group, and 2.36 ± 2.38 and 3.10 ± 1.31 mg/kg for the CdCys group as compared to 0.04 ± 0.02 and 0.05 ± 0.02 in the control group. Also in the low dose groups, levels were increased, being 0.61 ± 0.09 and 0.98 ± 0.13 mg/kg for the CdCl₂ group, and 0.62 ± 0.17 and 0.37 ± 0.18 mg/kg for the CdCys group. In the high dose groups this was still the case after 5 or 9 weeks on clean feed, indicating that the increase is not only due to possibly remaining feed in the samples. Also in the ileum and jejunum levels in the high dose groups were clearly increased compared to the controls (Table S2).

Hair samples were also collected at the time of slaughter but levels were very variable and it was difficult to draw conclusions (Table S1), especially due to a high level in one pig in the control group. When focussing on the pigs slaughtered after 12 weeks, levels in hair of the exposed animals are somewhat elevated but there is no clear dose-related increase. Overall, this questions the use of hair as an indicator of exposure, as was suggested by data from other studies (King et al. 1992, Phillips et al. 2003) although these studies also showed variable results.

Cadmium levels in blood

Concentration data of Cd in RBCs for the high dose groups are shown in Figure 7. For each of the two Cd forms, CdCl₂ and CdCys, the data showed similar results. All data together of the pigs exposed during 3 weeks to 10 mg Cd/kg feed were taken to fit the model parameters of the model equations (1) and (2). The model parameters thus obtained are the background concentration $C_0 = 0.11$ (SD = 0.08) µg/kg, the steady state elevation level $\Delta C_{ss} = 14.0$ (SD = 0.3) µg/kg and the dilution/elimination rate $k = 0.0272$ (SD = 0.0008)/day. The model was also verified on the data obtained for the animals with a prolonged exposure of 12 weeks. Figure 7 (left panel) shows a clear fit for the data obtained for the high dose groups exposed for 12 weeks, reaching a plateau after about 15 weeks. When stopping the exposure after 3 weeks, the levels decreased gradually and reached background levels after about 15 weeks.

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The model was also verified on the data obtained from the continued Cd administration experiment of 1 mg/kg feed (Figure 7; right panel). Verification was obtained by multiplication of ΔC_{ss} by the ratio 0.0875 of total applied Cd of the low dose and high dose regimen. Although most of the levels were around the LOQ for RBCs, the verifications show a fair comparison between the model and the data.

Conclusions

The present study shows the time- and dose-related increase in Cd levels in various tissues of growing pigs, in most cases in line with previous studies. The use of different exposure periods and the switch to clean feed allowed the modelling of the data, leading to a mechanistic model that can be used in future incidents with Cd but also helps to understand the behaviour of Cd in growing pigs. Following the switch to clean feed, the decrease in the levels in liver and kidneys could entirely be explained by the further growth of the animals.

There was no clear difference between the two applied forms of Cd, suggesting that at least these forms show similar ADME properties in pigs. Other authors fed pigs with plant materials grown on contaminated fields and observed levels in livers and kidneys that were in a similar range as observed with CdCl₂. Therefore, these studies do not indicate a difference in the bioavailability of different forms of Cd, assuming that most of the Cd was present in a bound form in the plant materials.

In domestic animals the largest fraction, of Cd is found in the liver and the kidneys. The present study confirms this finding, with Cd concentrations in the liver and the kidneys exceeding those in the rest of the body manifold. However, preferential Cd accumulation in the liver over the kidneys with increasing exposure (EFSA, 2004) was not found in the present study. To the contrary, the accumulation of Cd in the liver and the kidneys showed similar dynamics (see Figures 4 and 5), however with the concentration in the kidneys exceeding that in the liver by a factor of four. The latter effect is in concordance with the MT findings in these organs. Despite organ growth there was an increase of the MT level in the kidneys but a decrease in the MT level in the liver at increasing exposure (Figure 3). These findings indicate an independent role of the kidneys in Cd accumulation, i.e. kidney specific MT synthesis followed by Cd binding to the induced MT proteins. Alternatively, it has been suggested that the exposure to Cd may lead to the binding of Cd²⁺ to serum albumin which may be converted to the Cd-MT complex in the liver. Circulating Cd-MT complexes than

may reach the kidneys, where they may be filtered by the glomerulus and reabsorbed by the proximal tubule cells (EFSA, 2004). Though the results of the present study certainly do not exclude the latter disposition mechanism, as said, they also hint on the existence of a kidney specific Cd binding mechanism, i.e. the induction of kidney specific MT proteins followed by the binding of Cd in the blood to the induced proteins.

Overall, the current feed limit in the EU of 0.5 mg/kg prevents that levels in kidneys and livers exceed the limits, provided that the exposure period is limited to less than 12 weeks.

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Legends

Figure 1. Set-up of the animal study.

Figure 2. Body weights of control pigs, pigs exposed to 1 mg/kg or 10 mg/kg CdCl₂ or Cd-cysteine for 12 weeks, or pigs exposed for 3 weeks to 1 or 10 mg/kg followed by 9 weeks on clean feed. Since the body weights were similar for CdCl₂ or Cd-cysteine, data were combined (mean $\pm$ SD for n=3 (controls) or n=6 (exposed) except for the 10 mg/kg group for 12 weeks where n=5 due to 1 animal dropping out). a: significantly different from that of the combined control and low dosed pigs (p<0.05, Student t-test, two-sided).

Figure 3. Levels of metallothioneins in kidneys (upper left) and livers (upper right) of 1) control pigs, or pigs exposed to CdCl₂ or Cd-cysteine at 2) 1 mg/kg for 12 weeks, 3) 1 mg/kg for 3 weeks followed by 5 or 9 weeks on clean feed, 4) 10 mg/kg for 12 weeks or 5) 10 mg/kg for 3 weeks followed by 5 or 9 weeks on clean feed (3+9). Since the MT concentrations were similar for CdCl₂ and Cd-cysteine, data were combined (mean $\pm$ SD for n=3 (controls) or n=6 (exposed)). a,b: statistically significant different from control (a) or continued (b) exposure (p<0.05, two-sided Students t-test). Lower panels show the relation between concentrations of Cd and MT in kidneys (left panel, linear relation) and livers (right panel, constant).

Figure 4. Comparison of model for the absolute amount of Cd (line) and experimental data (closed circles) in kidney (left panel) and liver (right panel) after 3 and 12 weeks of feeding contaminated feed at a level of 1 mg/kg (lower line) and 10 mg/kg (upper line).

Figure 5. Comparison of the model for the concentration of Cd (line) and experimental data (closed circles) in kidney (left panels) and liver (right panels). Upper panels: 1 mg/kg feed contamination level; lower panels: 10 mg/kg feed contamination level.

Figure 6. Model simulation of the time-course of the Cd concentration in the kidneys (left panel) and the liver (right panel) after feeding contaminated feed at a level of 0.5 mg/kg (EU limit). Horizontal lines: EU limits for Cd in the kidneys (1.0 mg/kg wet weight) and liver (0.5 mg/kg wet weight).

Figure 7. Cd concentrations in red blood cells (RBC) of six animals, three on CdCl₂ and three on CdCys, both at about 10 mg Cd per kg feed. The model was fit to the data from animals

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622 ended after 12 weeks resulting in increased levels which served the model verification (upper
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624 Nevertheless, indicative values for lower exposure levels were reported and plotted too (right
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Table 1. Concentrations and absolute amounts of Cd in livers and kidneys of pigs (mean ± SD). The EU limits are 0.5 and 1 mg/kg respectively.

Dose (mg/kg feed)	Period		Liver		Kidney	
	(weeks)		Level (mg/kg)	Total (mg)	Level (mg/kg)	Total (mg)
	Cd	Clean				
Control	0	0	0.03 ± 0.01	0.013 ± 0.002	0.13 ± 0.05	0.009 ± 0.006
	0	3	0.01 ± 0.01	0.013 ± 0.005	0.09 ± 0.01	0.016 ± 0.009
	0	12	0.02 ± 0.01	0.030 ± 0.016	0.08 ± 0.03	0.033 ± 0.009
CdCl ₂ 1 mg/kg	3	0	0.10 ± 0.03	0.087 ± 0.024	0.44 ± 0.10	0.080 ± 0.019
	12	0	0.34 ± 0.07	0.660 ± 0.158	1.53 ± 0.21	0.651 ± 0.062
	3	5	0.13 ± 0.02	0.175 ± 0.035	0.51 ± 0.12	0.158 ± 0.034
CdCl ₂ 10 mg/kg	3	9	0.08 ± 0.03	0.150 ± 0.031	0.38 ± 0.13	0.153 ± 0.048
	3	0	1.03 ± 0.15	1.025 ± 0.116	5.00 ± 0.79	1.033 ± 0.233
	12	0	3.20 ± 0.26	5.620 ± 0.384	14.67 ± 1.15	5.444 ± 0.294
CdCys 1 mg/kg	3	5	0.76 ± 0.14	1.094 ± 0.149	3.53 ± 0.25	1.176 ± 0.195
	3	9	0.50 ± 0.09	0.855 ± 0.209	1.80 ± 0.14	0.808 ± 0.184
	3	0	0.12 ± 0.02	0.113 ± 0.009	0.46 ± 0.07	0.082 ± 0.009
CdCys 10 mg/kg	12	0	0.37 ± 0.05	0.724 ± 0.099	1.43 ± 0.15	0.652 ± 0.043
	3	5	0.09 ± 0.02	0.132 ± 0.024	0.39 ± 0.05	0.136 ± 0.021
	3	9	0.09 ± 0.02	0.158 ± 0.049	0.36 ± 0.15	0.148 ± 0.092
CdCys 10 mg/kg	3	0	0.94 ± 0.04	0.870 ± 0.073	4.60 ± 0.78	0.892 ± 0.199
	12	0	3.35 ± 0.07	5.409 ± 0.037	11.50 ± 0.71	4.259 ± 0.168
	3	5	0.76 ± 0.21	1.045 ± 0.344	3.23 ± 0.60	1.014 ± 0.228
CdCys 10 mg/kg	3	9	0.64 ± 0.19	1.182 ± 0.035	2.30 ± 0.36	1.013 ± 0.107

Supplementary files

Figure S1. Kidney and liver weights at 3, 8 and 12 weeks after the start of the experiment. Regression: kidney (lower line and symbols), weight at start of experiment 0.114 (kg), growth factor 0.00635 (kg/d); liver (upper line and symbols) weight at start of experiment 0.626 (kg), growth factor 0.0143 (kg/d).

Figure S2. Levels of Cd in livers (♦) and kidneys (●) of pigs treated with a low (A) or high (B) dose CdCl₂ for 3 or 12 months, or for 3 months followed by 5 or 9 months of clean feed (◇,○). Figures C and D show the absolute amounts of Cd in livers and kidneys. Note that the difference in organ concentrations is almost compensated by the organ weights.

Table S1. Feed consumption and feed conversion

Dose (mg/kg feed)	Period (weeks)		Feed consumption (kg/animal/day)			Feed conversion ratio*			Cadmium Intake (mg)
	Cd	Clean	0-3 wk	3-8 wk	8-12 wk	0-3 wk	3-8 wk	8-12 wk	
0	0	0							
	0	3	1.42			1.69			2
	0	12	1.27	2.03	2.70	1.79	1.82	2.59	8
CdCl ₂ 1	3	0	1.22			1.90			20
	12	0	1.33	2.01	2.84	1.86	1.75	2.69	154
	3	5	1.48	1.94		1.80	1.82		27
	3	9	1.49	1.64	2.71	1.79	1.73	2.78	30
CdCl ₂ 10	3	0	1.47			1.71			293
	12	0	1.27	1.92	2.25	1.98	1.84	3.17	1558
	3	5	1.59	2.41		1.72	2.12		320
	3	9	1.46	2.26	2.97	1.91	1.98	3.62	298
CdCys 1	3	0	1.27			1.77			27
	12	0	1.34	2.17	2.76	1.76	1.87	3.17	157
	3	5	1.40	2.11		1.68	1.96		32
	3	9	1.42	1.96	2.52	1.76	1.85	2.46	35
CdCys 10	3	0	1.26			1.63			249
	12	0	1.47	1.68	2.20	2.62	2.16	3.38	1491
	3	5	1.33	1.71		1.94	2.04		265
	3	9	1.37	2.13	2.47	1.72	2.03	2.66	277

*feed consumption/weight gain

Table S2. Body, liver and kidney weights of pigs in the various dosage groups (mean ± SD, n=3) as determined at the time of slaughter.

Dose (mg/kg feed)	Period (weeks)		Body weight (kg)	Liver weight (g)	Liver weight (%)	Kidney weight (g)	Kidney weight (%)
	Cd	Clean					
Control	0	0	20 ± 1	477 ± 56	2.4 ± 0.4	85 ± 71	0.60 ± 0.15
	0	3	38 ± 4	968 ± 245	2.6 ± 0.4	181 ± 93	0.48 ± 0.22
	0	12	97 ± 7	1974 ± 414	2.0 ± 0.3	429 ± 73	0.44 ± 0.04
CdCl ₂ 1 mg/kg	3	0	32 ± 2	851 ± 82	2.7 ± 0.1	184 ± 9	0.58 ± 0.02
	12	0	102 ± 8	1930 ± 84	1.9 ± 0.1	426 ± 21	0.42 ± 0.02
	3	5	65 ± 7	1319 ± 214	2.0 ± 0.1	312 ± 13	0.48 ± 0.03
	3	9	94 ± 5	1831 ± 253	2.0 ± 0.4	411 ± 32	0.44 ± 0.05
CdCl ₂ 10 mg/kg	3	0	37 ± 2	1010 ± 170	2.8 ± 0.4	206 ± 25	0.56 ± 0.01
	12	0	87 ± 3	1758 ± 70	2.0 ± 0.0	373 ± 35	0.43 ± 0.03
	3	5	71 ± 7	1457 ± 158	2.1 ± 0.2	331 ± 32	0.46 ± 0.00
	3	9	95 ± 8	1718 ± 103	1.8 ± 0.0	447 ± 67	0.47 ± 0.03
CdCys 1 mg/kg	3	0	35 ± 2	931 ± 95	2.6 ± 0.3	178 ± 6	0.50 ± 0.04
	12	0	97 ± 6	1938 ± 88	2.0 ± 0.2	456 ± 19	0.47 ± 0.02
	3	5	70 ± 6	1455 ± 168	2.1 ± 0.1	349 ± 45	0.50 ± 0.04
	3	9	100 ± 3	1767 ± 382	1.8 ± 0.3	399 ± 116	0.40 ± 0.11
CdCys 10 mg/kg	3	0	34 ± 3	927 ± 92	2.7 ± 0.2	194 ± 30	0.56 ± 0.10
	12	0	87 ± 1	1615 ± 45	1.8 ± 0.0	372 ± 37	0.43 ± 0.03
	3	5	59 ± 12	1365 ± 143	2.3 ± 0.3	313 ± 40	0.53 ± 0.08
	3	9	96 ± 9	1950 ± 588	2.0 ± 0.4	443 ± 22	0.46 ± 0.05

Table S3. Cadmium levels in lungs, testicles and hair of pigs (mean $\pm$ SD).

Dose (mg/kg feed)	Period		Lungs (mg/kg)	Testicles (mg/kg)	Hair (mg/kg)
	Cd	Clean			
Control	0	0	<0.010	<0.010	0.014 $\pm$ 0.007
	0	3	<0.010	<0.010	0.452 $\pm$ 0.536
	0	12	<0.010	<0.010	0.026 $\pm$ 0.010
CdCl ₂	3	0	<0.010		0.230 $\pm$ 0.203
1 mg/kg	12	0	0.021 $\pm$ 0.011	<0.010	0.108 $\pm$ 0.008
	3	5	<0.010	<0.010	0.005 $\pm$ 0.007
	3	9	<0.010	<0.010	0.078 $\pm$ 0.031
CdCl ₂	3	0	0.083 $\pm$ 0.032	0.020 $\pm$ 0.010	0.245 $\pm$ 0.042
10 mg/kg	12	0	0.230 $\pm$ 0.040	0.049 $\pm$ 0.007	0.120 $\pm$ 0.020
	3	5	0.052 $\pm$ 0.006	<0.010	0.011 $\pm$ 0.004
	3	9	0.049 $\pm$ 0.008	0.016 $\pm$ 0.008	0.083 $\pm$ 0.045
CdCys	3	0	<0.010	<0.010	0.118 $\pm$ 0.044
1 mg/kg	12	0	0.028 $\pm$ 0.009	<0.010	0.071 $\pm$ 0.040
	3	5	<0.010	<0.010	0.006 $\pm$ 0.001
	3	9	<0.010	<0.010	0.039 $\pm$ 0.023
CdCys	3	0	0.079 $\pm$ 0.002	0.023 $\pm$ 0.003	0.288 $\pm$ 0.173
10 mg/kg	12	0	0.167 $\pm$ 0.084	0.046 $\pm$ 0.026	0.192 $\pm$ 0.051
	3	5	0.068 $\pm$ 0.018	<0.010	0.023 $\pm$ 0.003
	3	9	0.035 $\pm$ 0.013	<0.010	0.029 $\pm$ 0.023

Table S4. Cadmium levels in duodenum, Ileum and Jejunum of pigs (mean $\pm$ SD).

Dose (mg/kg feed)	Period		Duodenum		Ileum		Jejunum	
	(weeks)		(mg/kg)		(mg/kg)		(mg/kg)	
	Cd	Clean						
Control	0	0	0.08	$\pm$ 0.05	0.05	$\pm$ 0.07	<0.01	
	0	3	0.04	$\pm$ 0.02	<0.01		<0.01	
	0	12	0.05	$\pm$ 0.02	<0.01		<0.01	
CdCl ₂ 1 mg/kg	3	0	0.61	$\pm$ 0.09	0.04	$\pm$ 0.04	0.06	$\pm$ 0.00
	12	0	0.98	$\pm$ 0.13	0.06	$\pm$ 0.05	0.13	$\pm$ 0.04
	3	5	0.11	$\pm$ 0.02	<0.01		<0.01	
	3	9	0.09	$\pm$ 0.09	0.02	$\pm$ 0.02	<0.01	
CdCl ₂ 10 mg/kg	3	0	4.30	$\pm$ 2.80	0.25	$\pm$ 0.06	0.31	$\pm$ 0.02
	12	0	1.12	$\pm$ 0.17	0.45	$\pm$ 0.17	0.92	$\pm$ 0.63
	3	5	0.20	$\pm$ 0.06	0.03	$\pm$ 0.01	0.06	$\pm$ 0.01
	3	9	0.13	$\pm$ 0.01	0.04	$\pm$ 0.01	0.04	$\pm$ 0.00
CdCys 1 mg/kg	3	0	0.62	$\pm$ 0.17	0.04	$\pm$ 0.03	0.06	$\pm$ 0.02
	12	0	0.37	$\pm$ 0.18	0.05	$\pm$ 0.02	0.12	$\pm$ 0.02
	3	5	0.06	$\pm$ 0.03	<0.01		<0.01	
	3	9	0.05	$\pm$ 0.02	<0.01		<0.01	
CdCys 10 mg/kg	3	0	2.36	$\pm$ 2.38	0.37	$\pm$ 0.25	0.32	$\pm$ 0.15
	12	0	3.10	$\pm$ 1.31	0.23	$\pm$ 0.13	0.37	$\pm$ 0.10
	3	5	0.18	$\pm$ 0.05	0.05	$\pm$ 0.02	0.07	$\pm$ 0.01
	3	9	0.11	$\pm$ 0.06	0.02	$\pm$ 0.01	0.14	$\pm$ 0.19

Table S5. Carry-over studies with cadmium in pigs.

Source	Weight (duration)	Levels (mg/kg)			Author, year
		feed	liver	Kidneys	
Corn, sewage sludge	18-90 kg	0.08/0.24	0.13/0.21	0.64/1.50	Lisk et al. 1982
Barley, municipal waste	(32–73 d)	0.05/0.23	0.17/0.33	0.78/1.39	Bache et al. 1987
CdCl ₂ , rock phosphate various	8-90 kg 3 (11 wks - 5-6 mo)	0/0.44/ 0.89 /2.21/4.4 0.60/1.20 0.037	0.30/0.30/ 0.38 /0.94/2.0 1 0.30/0.35	0.39/1.23/ 2.17 /5.73/12.2 5 1.13/1.68 0.04-0.11	King et al. 1992 Lindén et al. 1999
CdCl ₂ /barley Effect phytase, calcium Cd(NO ₃) ₂	17-30 kg 17-50 kg (80 g/kg bw)	0.03/ 0.78 (80 g/kg bw)	0.05/ 0.50/0.62/0.48/0.4 7 0.05/0.66/0.95/0.69/0.6 3	0.6/ 4.5/6.9/4.6/4.5 1.1/7.2/11.7/7.9/6.7	Zacharias et al. 2001
CdCl ₂	17-97 kg (137 d)	0/0.5/1.0/2.5	0.02/0.03/0.07/0.16*	0.05/0.10/0.42/0.60	Phillips et al. 2003
CdCl ₂	(28-89 983 d)	0.11/10	0.1/6.9	0.2/13.9 (medulla) 0.4/33.0 (cortex)	Xu et al. 2004
CdCl ₂	28-90 kg (83 d)	0/0.5/5/10	nd	Nd	Tu et al. 2007
CdCl ₂ , CdCys	20-35 kg 20-97 kg	0.06/ 0.8 /10	0.01/ 0.11 /0.99 0.02/ 0.36 /3.28	0.13/ 0.45 /4.80 0.08/ 1.48 /13.1	This study

*estimated from figures