Cadmium from Soil Amended with Sewage Sludge: Effects and Residues in Swine
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Introduction

Municipal sewage sludge, the product of secondary and tertiary wastewater treatment, presents an increasingly burdensome disposal problem to sanitary districts of all sizes. Surface land disposal is an attractive alternative, but there are some potential hazards. Accumulation of toxic trace elements is the current primary concern and cadmium is the trace element receiving the most attention. Trace element composition of digested sewage sludges is highly dependent on the city of origin and on the metal processing activities within the recipient sanitary district (1). Heavy sewage sludge application to agricultural soils for several years results in significantly increased concentrations of nutrient elements, increased organic matter and moisture holding capacity, and does not adversely affect microbial action (2). Increased productivity is possible, especially on devastated soils or when suboptimum levels of commercial fertilizers are used (3). Sewage sludge applications also result in the accumulation of significant amounts of trace elements in the soils (2-4).

The effects of sludge amendment on crops include a frequent enhancement of production and increases in nutrient elements and nonessential trace elements in plant tissues (4-6). Plant leaves accumulate higher metal concentrations than do grains. Incorporation of previously applied sludge into the soil immediately prior to planting seems to make the metals more available than with an even heavier soil load from long term cumulative applications.

Feeding plant material grown on soils heavily fertilized with sewage sludge to animals has not elicited any definitive toxicity syndrome; however, there have been accumulations of potentially toxic trace elements in animal tissues, especially in kidney but rarely in muscle (6-8). The presence and accumulation of potentially toxic elements makes it desirable to conduct more intensive investigations for possible effects. We have generated residue data not available when a previous swine feeding study was published (8), and this has necessitated re-evaluation of some conclusions. In addition, a longer-term swine reproduction study involving more intensive exposure has recently been completed and some of these data will also be evaluated.

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Materials and Methods

Clinical and Analytical

Complete blood cell counts, serum enzymes and electrolytes, histopathology, electroencephalography, and electrocardiography were conducted using standard techniques. Metal concentrations were determined by atomic absorption spectroscopy after wet and/or dry ashing the samples. Hepatic microsomes were isolated from perfused liver sections by differential centrifugation and assayed as previously described (9, 10).

Grain Feeding Study

Nine weanling crossbred (Hampshire/Yorkshire) gilts were randomly assigned to one of three slot-floored pens (1.2 × 4.7 m) equipped with automatic waterers. After a 2-week acclimation period on full feed, the gilts were limit-fed a standard growing ration according to one of the following regimens: "control" received a ration formulated with control corn for 56 days; "14-day" received a ration formulated with sewage sludge-fertilized (SF) corn for 14 days and the control ration for 42 days; "56-day" received the SF ration for the full 56 days. The SF corn was pooled from experimental plots and incorporated as 79.4% of the ration (8). Weekly weight and total feed consumption were recorded and various clinical parameters were monitored periodically. The swine were sacrificed at the end of the 56 day feeding period.

Winter Foraging

Sixteen weanling purebred Berkshire gilts were assigned to experimental corn plots in a random fashion, except that no siblings were permitted within the same dosage group. Each 6.1 × 12.2 m plot had received fractions (0, ¼, ½, or 1) of the maximum amount of sludge which could be applied, dependent on weather, during each of eight growing seasons. Additional sludge applications occurred between winters. The sludges applied to these plots were from the Chicago Southwest plant which received a high proportion of industrial effluent and the Cd content averaged about 200 ppm dry weight.

The plots were divided between the two buffer rows and individual shelters, feeders and waterers were provided. The gilts were introduced to the plots November 4, 1975 and removed March 20, 1976. They were bred by one of two Berkshire boars that spring, farrowed in October and returned to the same plots as sows December 2, 1976. They were again bred in January while on the plots, removed from the plots March 15, 1977 and farrowed in spring 1977. The sows were killed for examination after weaning the second litter.

Results and Discussion

Grain Feeding

Table 1 presents selected metal concentrations in the control and sludge-fertilized (SF) corn. The SF corn also contained 1.14 times more Kjeldahl nitrogen than did the control (NPK-fertilized) corn resulting in calculated protein concentrations of 16.5% for the control diet and 17.7% for the SF diet (8). The swine fed SF corn for the full 56 days performed slightly better than the other two groups, possibly due to the limit-feeding and slightly higher nutritive elements in the SF diets (8). Relative organ weights, microscopic pathology, clinical chemistry, cardiac function, and central nervous function showed no dose related differences.

The swine fed SF corn diets accumulated significantly more Cd in the liver and kidney than did controls, but hepatic Fe and renal Mn were lower than controls (Table 2). There were no significant changes in Cd, Cu, Fe, Mn, or Zn content in spleen, muscle, brain, or bone. The Fe decrease, also seen as a trend in the kidney, may be related to the Cd interference with Fe absorption described by Freedman and Cousins (11), since the SF corn contained a

| Metal | Control (ppm dry wt) | Sludge-fertilized (ppm dry wt) | Ratio SF/Control |
|-------|---------------------|-------------------------------|-----------------|
| Cd    | 0.10                | 0.56                          | 5.6             |
| Ca    | 23.48               | 26.65                         | 1.1             |
| Cu    | 1.99                | 2.22                          | 1.1             |
| Fe    | 14.23               | 18.45                         | 1.3             |
| Mn    | 7.40                | 6.98                          | 0.9             |
| Ni    | 0.66                | 1.28                          | 1.9             |
| Zn    | 26.8                | 31.9                          | 1.2             |

Table 2. Mean metal concentrations.

| Metal concn. (ppm fat-free dry weight) |
|---------------------------------------|
| Liver - Control                       | 0.19 | 616 | 12.5 | 0.66 | 168 | 7.7 |
| Liver - 14-day                        | 0.27 | 596 | 13.1 | 0.78 | 144 | 6.8 |
| Liver - 56-day                        | 0.32 | 409 | 13.0 | 1.41 | 138 | 6.4 |
| Kidney - Control                      | 0.08 | 163 | NS   | 0.42 | NS  | 0.8 |
| Kidney - 14-day                       | 0.31 | 167 | NS   | 0.42 | NS  | 0.8 |
| Kidney - 56-day                       | 0.53 | 167 | NS   | 0.42 | NS  | 0.8 |

a Least significant difference at p < 0.05; NS = not significant.
higher Fe concentration (Table 1).

A possible correlation may exist between the lower tissue Fe and some minor, but significant, differences in red blood cells (RBC). At 56 days, the pigs which had received SF corn had slightly lower packed cell volumes (PCV) and hemoglobin (Hb) values (Table 3). The red cell counts were lower for the SF fed pigs than for the control animals resulting in a significant increase of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). The 56 days of slightly decreased Fe absorption would probably not be adequate for the pigs to develop a significant iron deficiency anemia, as the life span of porcine erythrocytes is 85 ± 11 days. At higher Cd concentrations, however, Cousins et al. (12) produced pigs with decreased packed cell volumes in 42 days. At the low level of ‘biological’ Cd fed in our study, packed cell volumes were not depressed because of an apparent increase in the volume of erythrocytes (MCV). This slight change in the MCV may be attributed to a release of reticulocytes in response to minimal anemia. However, the fact that the mean corpuscular hemoglobin in concentration (MCHC) for all groups was essentially the same, indicates the possibility that the enumeration of erythrocytes (the most variable of the measurements) was in error. A more intensive survey of the effects of the higher metal content of sludge-fertilized grains on hematology is necessary.

The pigs receiving SF corn for 56 days had decreased hepatic microsomal O-dealkylation activity, another possible consequence of interference with iron metabolism. It was previously erroneously reported that this activity was enhanced (8). The swine fed SF corn for only 14 days did not differ from the controls, but the 56-day feeding resulted in higher microsomal protein concentrations but lower and more variable activity (Table 4). The 56-day group has a lower affinity for low substrate concentrations and a lower maximum reaction velocity V for high substrate concentrations (Table 5 and Fig. 1).

Although this type of biphasic plot is expected when a wide range of substrate concentrations are used (9, 13) the shape of the plot for swine liver microsomal metabolism of p-nitrophenetole is unusual (10). The low-substrate portion of the plot extrapolates to a higher V_max and is not as sensitive to CO inhibition as the high-substrate (low affinity) "enzyme." The factor(s) in SF corn which depress the microsomal activity maintain the general pattern even though slopes and intercepts are somewhat different.

Direct feeding of large amounts of sewage sludge (50% of diet) to male rats resulted in enhanced pentobarbital metabolism and liver enlargement (14). High levels of dietary cadmium have been shown to enhance microsomal enzymes and to

### Table 3. Hematological parameters at 56 days in swine fed sewage sludge-fertilized corn for 0, 14 or 56 days, (mean ± SD for n = 3).

| Group | RBC × 10⁶ | Hb, g-% | PCV, % | MCV μ³ | MCH × 10⁶, g | MCHC, % |
|-------|-----------|---------|--------|---------|---------------|--------|
| Control | 9.21 ± 0.74 | 14 ± 1 | 42.5 ± 2.7 | 45 ± 2 | 15 ± 1 | 33 ± 1 |
| 14 Day | 7.97 ± 0.66 | 13 ± 1 | 41.9 ± 2.0 | 53 ± 2b | 17 ± 1b | 32 ± 1 |
| 56 Day | 7.63 ± 0.44 | 13 ± 1 | 41.2 ± 1.4 | 54 ± 1b | 17 ± 1b | 32 ± 1 |

* Significant different from control at p < 0.05.

* Significant different from control at p < 0.01.

### Table 4. Hepatic microsomal O-dealkylation of p-nitrophenetole (substrate) in swine fed sewage sludge fertilized corn for 0, 14, or 56 days (mean ± SD for n=3).

| Group | Microsomal protein, mg/100 mg fw | Specific activity, moles p-nitrophenol produced/ min/mg protein |
|-------|---------------------------------|---------------------------------------------------------------|
|       | 0.05M substrate 0.25M substrate |

| Group | V, mole substrate/min-mg | K_m × 10⁶, M⁰ | γ⁵ | 0.0125-0.05M substrate | V, mole substrate/min-mg | K_m × 10⁶, M⁰ | γ⁶ |
|-------|--------------------------|---------------|-----|------------------------|--------------------------|---------------|-----|
| Control | 12.90 | 3.5 | 0.989 | 8.94 | 1.2 | 0.820 |
| 14 day | 12.90 | 2.9 | 0.998 | 9.69 | 1.3 | 0.882 |
| 56 day | 14.70 | 11.1 | 0.994 | 5.08 | 1.1 | 0.952 |

* Substrate concentration for ½ maximum velocity.

* Regression coefficient.
potentiate microsomal inducing agents (15), while peritoneal administration of cadmium depresses drug metabolism (16). Furthermore, several metals, including Cd, Cu, Fe, Ni, and Zn, induce microsomal heme oxygenase which degrades free heme as well as cytochrome P-450 (17). It is unlikely that the low concentration of Cd in the SF corn acted directly to depress the microsomal mixed function oxidase system. It is probable that an interaction between depressed Fe levels, increased Cd and/or increased dietary protein resulted in the increased synthesis of microsomal protein not related to drug metabolism and/or increased degradation of heme decreasing the metabolic potential of the protein present; thus, the increase in microsomal protein would not be accompanied by an increase in microsomal O-dealkylation activity. The question of Cd influence on microsomal drug metabolism presents some interesting paradoxes and should be a fertile area for future investigation.

Winter Foraging

Subjective evaluation of holes and wallows indicated that the swine on the maximum treated plots tended to root less than the other groups. In order to more precisely evaluate exposure through inges-

Table 6. Mean metal residues in soils amended for 8 years with sewage sludge and in the feces and kidneys of sows winter-foraging on these soils.

| Treatment group | Metal concentration ppm |
|-----------------|-------------------------|
|                 | Cd  | Cu  | Cr  | Ni  | Zn  |
| Control         |     |     |     |     |     |
| Soil*           | 4   | 0.68| 18  | 37.5| 16.6| 82  |
| Feces*          | 2   | 0.59| 22  | 4.4 | 3.6 | 225 |
| Kidney*         | 4   | 0.35| 5.3 | <0.5| <0.2| 27  |
| 1/4 Max         |     |     |     |     |     |
| Soil            | 4   | 4.59| 41  | 76.9| 22.5| 159 |
| Feces           | 3   | 0.49| 23  | 5.3 | 3.1 | 235 |
| Kidney          | 4   | 1.90| 3.7 | <0.5| <0.2| 28  |
| 1/2 Max         |     |     |     |     |     |
| Soil            | 4   | 9.90| 72  | 129.4| 27.6| 265 |
| Feces           | 4   | 0.85| 25  | 8.2 | 3.6 | 228 |
| Kidney          | 4   | 3.69| 5.5 | <0.5| <0.2| 30  |
| Maximum         |     |     |     |     |     |
| Soil            | 4   | 19.43| 122| 228.2| 37.0| 435 |
| Feces           | 4   | 7.19| 55  | 67.0| 12.1| 286 |
| Kidney          | 4   | 5.18| 6.4 | <0.5| <0.2| 31  |

* Spring 1976: ppm dry weight.
* February 19, 1977: ppm wet weight.
* Summer 1977: ppm wet weight.

Table 7. Representative concentrations of Cd and Cr in the feces of sows winter-foraging on sewage sludge amended corn plots.

| Group and soy no. | Metal concentration, ppm wet wt. |
|-------------------|----------------------------------|
|                   | Cadmium                         | Chromium                       |
|                   | 19 Feb 15 Mar* 20 Mar           | 19 Feb 15 Mar* 20 Mar           |
| Control           |                                 |                                 |
| 9-2               | 0.47                            | 1.15                           | 0.22                          | 6.1  | 9.1  | 1.3  |
| 11-1              | 0.72                            | 0.30                           | 0.22                          | 2.7  | 5.4  | 1.2  |
| ¼ Max             |                                 |                                 |
| 10-6              | 0.32                            | NS*                            | 0.30                          | 2.8  | NS   | 2.1  |
| 12-3              | 0.57                            | NS                             | 0.19                          | 5.9  | NS   | 1.0  |
| ½ Max             |                                 |                                 |
| 11-7              | 0.80                            | 1.58                           | 0.13                          | 9.0  | 12.8 | 1.4  |
| 12-7              | 0.90                            | 1.42                           | 0.25                          | 7.4  | 8.2  | 1.3  |
| Maximum           |                                 |                                 |
| 13-1              | 10.60                           | 9.01                           | 0.23                          | 83.2 | 82.2 | 1.4  |
| 14-7              | 5.79                            | NS                             | 0.35                          | 57.7 | NS   | 2.0  |

* Removed from experimental corn plots on 15 March, 1977.
* No adequately discrete sample was collected.

Table 8. Weight changes in sows winter foraging on sludge-amended soils for two years and two reproduction cycles.

| Group        | Mean weight ± S D kg |
|--------------|----------------------|
|              | Start | Breed | 1st Wean | 2nd Wean |
| Control      | 19.4 ± 0.2 | 102 ± 10 | 153 ± 17 | 179 ± 11* |
| ¼ Max        | 18.5 ± 2.4 | 103 ± 10 | 175 ± 9 | 205 ± 13 |
| ½ Max        | 19.4 ± 0.2 | 96 ± 14 | 166 ± 26 | 176 ± 10* |
| Maximum      | 20.1 ± 1.1 | 100 ± 4 | 144 ± 15 | 165 ± 21* |

* Significantly lower than ¼ max weights by Tukey's multiple means test at p = 0.007.
* Significantly lower than ¼ max at p < 0.003.
* Significantly lower than ¼ max at p = 0.0005.
tion, a series of fecal samples were collected and analyzed for heavy metals. Samples collected in February 1977, while the ground was frozen and moderately snow-covered, indicated that there was a dose related increase in fecal metal residues, but this was not necessarily directly related to propensity to root. The relationship between soil, fecal and renal residues of selected metals is shown in Table 6. Cadmium was the only metal which accumulated significantly in the kidney tissue. Soil ingestion increased as spring approached and the ground thawed, but residues of metals in the feces declined rapidly once the swine were removed from the plots (Table 7).

Complete data for residues and biological effects are not yet available. Pigs started out uniform and gained weight at a reasonably uniform rate up to maturity (Table 8); however, after weaning the first litters a trend started in which the animals on maximum treated plots seemed to suffer slightly from the heavy sludge application rates. The swine foraging on plots amended with ¼ the maximum application rate responded more favorably to the stress of reproduction and ultimately gained significantly more weight than any of the other groups (Table 8).

The first year's farrowing records cannot be taken too literally. Some gilts had unusually traumatic first parturitions and, in fact, one from the maximum treated group died from massive hemorrhages suffered during delivery. To make the comparisons more precise, only sows which farrowed both times are included in the data: the sow that died in the maximum treated group is not included, but had 6 liveborn pigs; the replacement for this sow is not included (11 liveborn piglets and 4 fetal deaths); one sow each from the control and ¼ maximum groups did not conceive the second time in spite of multiple attempts at breeding.

The swine in the control and maximum treated groups seemed to be slightly more fertile than the two intermediate groups, both as gilts and as sows (Table 9). The group foraging on plots amended with ½ the maximum possible sewage sludge application for two winters generally conceived fewer pigs, but all of these were born alive and fully developed (Table 10). These data must be tempered with an appreciation for the low experimental numbers and the high degree of variability. The number of stillborn piglets is exaggerated, for example, in the first-litter ¼ maximum group and the second-litter maximum group by single sows, which had breech birth complications resulting in 5 and 6 stillborn piglets, respectively. The number of piglets not fully developed is more closely related to total litter size than to treatment group; all but one of the 17 fetal deaths occurred in litters totaling 12 pigs or more. Thus, it is equally as likely that the excellent in-utero survival rate of ½ maximum piglets is due to lower fertility as to a positive influence of nutrient factor(s) in the sludge. On the other hand, the fact that gilt-to-sow performance improved and there were no piglet deaths indicates that this rate of sewage sludge application does not render these soils toxic to foraging swine.

Although there is a direct relationship between treatment level and Cd residues, there were no significant differences in the iron content of treated and untreated soils. This is not surprising, since the iron content was near 2% in the soil and the sludge contribution would be minimal. However, acid extractable (bioavailable) Fe is significantly increased in sewage sludge amended soils and the pH of the soil is decreased (Table 11). Even though there is a direct relationship between treatment level and available soil Fe, tissue Fe does not increase linearly (Fig. 2). Thus, swine store the increased available Fe at lower sludge application rates, but as the rate of sludge application increases the Fe stores and blood hemoglobin decline again (Fig. 2). This

| Group    | $n$ | 1st Litter (10/76) | 2nd Litter (4/77) |
|----------|-----|-------------------|-------------------|
|          |     | Liveborn          | Conceptions$^a$   | Liveborn          | Conceptions$^a$   |
| Control  | 3   | 7.7 ± 0.6         | 10.3 ± 1.2        | 9.3 ± 1.1         | 11.7 ± 3.5        |
| ¼ max    | 3   | 7.3 ± 2.3         | 9.7 ± 2.5         | 7.0 ± 2.6         | 8.3 ± 3.2         |
| ½ max    | 4   | 7.0 ± 1.8         | 8.8 ± 2.2         | 7.8 ± 3.4         | 7.8 ± 3.4         |
| Maximum  | 3   | 9.0 ± 2.0         | 10.7 ± 1.5        | 7.7 ± 4.2         | 12.3 ± 3.5        |

$^a$ Total liveborn + stillborn + not full term (i.e., fetal deaths).
may be related to the increased Cd which interferes with Fe absorption and/or to some of the many other factors in the sewage sludge. It appears that soil concentrations of 4 or 5 ppm Cd would not interfere with Fe absorption in swine ingesting the soils, but at higher Cd concentrations, it is more difficult for the swine to take advantage of the increased available Fe. Again, it would be desirable to investigate these relationships in more detail.

In summary, the swine foraging on moderately heavily amended plots (¼ and ½ maximum) frequently outperformed the animals on control or maximum treated plots. This pattern persisted for weight gain, in-utero piglet survival, blood hemoglobin, and tissue iron concentrations, even though the diet was standard and nutritionally adequate. At the lower Cd intake of the grain-feeding study there was also a modest performance advantage of the SF corn, but this could have been due to higher nutritive value, since these pigs were limit-fed. Only at very high sewage sludge application rates did there appear to be signs of toxicity, and these were generally significant only when compared to intermediate application rates, essentially the same as controls. Nevertheless, the modest performance advantage gained must be balanced against the increased Cd residues even though these increases are not observed in muscle tissue. Since the swine foraging study probably represents maximum animal exposure to land applied sewage sludge, it suggests that moderate agricultural use should present little direct toxicity hazard; however, the data are scant and present some apparent anomalies. Reliable risk-benefit analysis is impossible at this time with only these data.

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