Introduction

Thallium (Tl) is a metal in the boron group (IIIA) of the periodic table. It is a commonly found element in the natural environment, but in a highly dispersed form. The mean content of thallium in the earth crust is in the range of 0.1-1.7 mg/kg. This chemical element coexists with sulfur ores of heavy metals as zinc, copper, iron or lead [1-5]. Other thallium minerals such as crookesite (Tl, Cu, Ag)2Se, loran-dite TlAsS2, and hutchinsonite (Tl, Ag)2Pb(AsS2)4 (with thallium content of over 60%) are very rare in nature [6-8]. Thallium was scored to the group of trace elements with the highest accumulation factor in the environment, in addition to elements such as mercury, cadmium, lead, zinc, and chromium. Increased levels of this element in the natural environment are mainly correlated with anthropogenic sources [9-11] and the estimated half-life in humans is up to 3 days [31]. According to American Conference of Governmental Industrial Hygienists (ACGIH) and Occupational Safety and Health Administration (OSHA) estimations, permissible concentrations of thallium in confined spaces may oscillate up to 0.1 mg/m³ [8, 12, 13]. Typically, metal toxicity varies among species, metals, and physico-chemical conditions [19].

In the farm animal species, the heavy metal studies and its bioaccumulation and concentration in the organisms and health implications are still not well known. Moreover, there are limited materials in this field in scientific sources. Therefore, the aim of this study was to determine the influence of chronic thallium intoxication (in experimental con-

Abstract

Thallium is a metal with a high rate of accumulation in the environment and at the same time there is a very large dispersion of it. Heavy metal studies and its bioaccumulation and concentration in organisms and health implications are still not well known. The aim of this study was to demonstrate the importance of this element as an intoxicant of living organisms, especially on laying hens. Four groups of ISA Brown hens were used in this study. Experimental groups received feed containing thallium as thallium sulfate (Tl₂SO₄). Concentrations and metal accumulation in the animals were determined periodically, analyzing the eggs and animal blood. After the experiment, concentrations of thallium in the tissues was determined post-mortem. The studies showed a proportional accumulation of thallium in the body of the animals in relation to exposure time, and doses with the highest concentrations in the bones, kidneys, muscles, liver, and blood.

Keywords: thallium, intoxication, laying hens, blood

The Influence of Chronic Thallium Intoxication on Laying Hens, Including Its Cumulation in Tissues, Organs, and Eggs

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Abstract

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Materials and Methods

Studies on the effect of different doses of thallium in the body of ISA Brown laying hens was carried out in a vivarium of the Department of Environmental Hygiene and Animal Welfare at Wrocław University of Environmental and Life Sciences. All the experimental procedures were reviewed and accepted by the 2nd Local Ethics Committee (Wroclaw, Poland). The age of the laying hens at the start of the experiment was 52 weeks, after two weeks of adaptation to the caging system. A total of 64 animals were used in this study. The laying hens were divided into 4 groups of 16 animals. The first group, the control (I), was fed by a standard feed, the second group (II) was fed with the addition of 2.6 mg Ti/kg, the third group (III) with the addition of 8 mg Ti/kg, and fodder in the fourth group (IV) contained the addition of 16 mg Ti/kg. Mentioned thallium concentrations in groups II, III and IV corresponded to a daily intake dose of thallium -0.14, 0.38, and 0.74 mg/kg/day, respectively. The content of thallium in fodder with daily intake was presented in Table 1. The experiment was carried out over 8 weeks. The birds were kept in the battery system, two laying hens per cage. The environmental conditions (temperature, lighting, ventilation) in the experimental room met the requirements for laying hens. Hens were fed with a standard feed for laying hens (DJ). The feed for the experimental groups (II, III, IV) contained the thallium as thallium sulfate (Tl₂SO₄). During each experiment the eggs as well as the blood samples were collected for testing at days 1, 14, 28, 42, and 56 of the experiment. At day 56 of the experiment the birds were slaughtered and the samples were taken from the following tissues and organs: liver (right lobe of liver – lobus dexter hepatis), kidney (renes), breast muscle (musculus pectoralis major), thigh muscles (biceps femoris – musculus biceps femoris), and thigh bone (femur stem – diapysis os femori). Blood samples were collected in tubes containing EDTA (Arabinda) from the basilic vein (vena basilica). The experiments were performed with the quantitative analysis of thallium in the blood, its content in the eggs, liver, kidney, breast muscle, thigh muscle, and femur, and markings and haematological, and biochemical parameters: the number of red blood cells, white blood cells, haemoglobin, and haematocrit, as well as the activity of the glutamic oxaloacetic transaminase (GOT) and the glutamic pyruvic transferase (GPT) enzymes were determined by kinetic methods using Cormay reagents [16]. Creatinine levels were determined by photocolorometric method [16]. Determination of the haemoglobin was made by cyanidmethaemoglobin methode [17]. A spectrophotometric camera (UV-VIS Semco S.A.) was also used. Determination of hematocrit was performed using a centrifuge hematocrit [15]. The content of Na⁺, K⁺, and Cl⁻ in the serum was determined using ion-selective electrodes (ISE) in the Ciba-Corning 865 apparatus. The mean values were calculated (x) as well as the standard deviation (SD), significant differences between the control and experimental groups and the significant differences between experimental groups. Statistically significant differences (p ≤ 0.05) and highly significant differences (p ≤ 0.01) were performed with the analysis of variance and the results were verified by Duncan’s test. Moreover, the value of the correlation coefficient (r) between the thallium content in the feed and the concentration of this element in tissues and hen organs was calculated, similarly to the concentrations of thallium in various tissues and hen organs. Statistical analysis was performed using STATGRAPHICS ver. 5.0 and Microsoft Excel 2007.

Results

The Thallium Content in Blood

The analysis of thallium concentration in the blood at various stages of the presented study showed an increase trend in the content in all experimental groups (Group II-IV). The mean content of thallium in the hens blood in the control group (I) was 0.34 mg/l. In experimental groups II, III, and IV, intoxicated with increasing doses of thallium, the mean content of this element in the blood reached 59.66, 157.66, and 248.87 mg/l, respectively. The largest increase of thallium concentration in the blood, in all experimental groups, was during the first period of the study (days: 1-14). In these groups, after day 15 of the experiment, the thallium content in the blood continued the increase trend, but not so sharply. On the last day of the experiment in group IV (with the highest intoxication dose), the thallium concentration was 287.87 mg/l, which was nearly 800 times higher than before intoxication. The thallium content in the blood, in each experimental group (II-IV), significantly increased (p ≤ 0.01) together with increasing doses of thallium in the feed. The value of the correlation coefficient (r) between the thallium content in the feed and in the blood was very high and amounted up to 0.9116 (p ≤ 0.01). The content of thallium in hens blood as well as in tissues and eggs was presented in Table 2.

The Content of Thallium in Tissues

The mean content of thallium in the breast muscles in laying hens in the control group (I) was 0.013 mg/kg. The level of thallium in breast muscles increased in each...
The Influence of Chronic Thallium...

Table 1. The content of thallium in fodder with daily intake.

<table>
<thead>
<tr>
<th>Group</th>
<th>Thallium content in fodder</th>
<th>Mean daily intake [g/capita]</th>
<th>Daily thallium dose [mg/kg body weight]</th>
<th>Total thallium consumption during the experiment [mg/kg body weight]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>119.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>2.6</td>
<td>116.3</td>
<td>0.1374</td>
<td>7.7</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>105.7</td>
<td>0.3834</td>
<td>21.52</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
<td>101.1</td>
<td>0.7353</td>
<td>41.18</td>
</tr>
</tbody>
</table>

Experimental group (II, III, and IV), together with an increased content of this element in the feed. The highest content of thallium in the breast muscle was observed in group IV \( (p \leq 0.01); \) it was 2.70 mg/kg, more than 200 times higher than the concentration of thallium control group. Similar results were observed in the thighs. The content of thallium in thigh muscle in group I was 0.011 mg/kg, but in the experimental groups the value increased with increasing doses of thallium in the feed. The highest concentration of thallium in the thigh muscle was also observed in group IV, which was intoxicated with the highest dose of this element \( (p \leq 0.01); \) it was 2.954 mg/kg, which was nearly 270 times higher than in the control group. The value of the correlation coefficient \( (r) \) between the dose of thallium in the feed and the content of the element in the muscle was high and for the breast muscles was 0.9887 and 0.9875 for the thigh muscles \( (p \leq 0.01). \) The organ accumulating the smallest amounts of thallium was the liver. The contents of this element in the liver in the control group were similar to other organs, and was 0.014 mg/kg. The content of thallium in the experimental groups in the liver increased with increasing doses of thallium in feed, which was the reason that its peak in group IV was 1.452 mg/kg.

The value of the correlation coefficient \( (r) \) between the thallium concentrations in the feed and the content of the liver was 0.9975 \( (p \leq 0.01). \) The content of thallium in the kidneys as well as in other tissues increased in all of the experimental groups with increasing doses of thallium in the feed. The range of this content was 0.018 mg/kg in the control group (I), up to 7.432 mg/kg in group IV. The level of thallium in the kidneys in group I was lower only when it was compared with the thallium level in the bones. The level of thallium in the bones was growing in all experimental groups (II-IV) compared to the control \( (p \leq 0.01). \) This content was 0.022 mg/kg and was more than 430 times lower than the content of group IV \( (9.496 \text{ mg/kg}). \) The value of the correlation coefficient \( (r) \) between the thallium content in the feed and the content in the kidneys and bones was high at the level of 0.9826 and 0.9969, respectively \( (p \leq 0.01). \) In conclusion, the thallium concentration was significantly higher in the group intoxicated with higher doses of thallium. Among the examined organs and tissues the highest concentrations of thallium in all groups were found in the bones, and then in the kidneys, muscle and the lowest values in the livers. The value of this ratio was very high, with a range 0.8437 up to 0.9981 \( (p \leq 0.01). \) The lowest value of the correlation coefficient was observed between the thallium content in the blood and the concentration in the thigh muscle, and the highest between the content of this element in the femur and in the liver.

The Content of Thallium in Eggs

The thallium level in the hen control group, in the entire experiment, ranged from 0.010 up to 0.026 mg/kg. In experimental groups with thallium intoxication (II, III and IV) an increase content of this element in eggs was observed. The content of thallium in the eggs in II group increased from 0.12 mg/kg at the first day to 0.543 mg/kg on the final, 56th day of the experiment. In group III, the thallium content increased from 0.019 to 1.699 mg/kg during the experimental procedure. The highest concentrations of thallium in eggs were observed in group IV, where the level of this element increased from 0.015 mg/kg (first day of intoxication) up to 3.287 mg/kg on the 56th day of the experiment. During the experiment the contents of thallium in eggs were higher in the groups receiving fodder with a higher content of this element. The differences in the concentration of thallium in eggs between groups in different experimental periods were statistically highly significant \( (p \leq 0.01). \) The changes in the content of thallium in eggs, depending on the time and the thallium concentration in the feed, were shaped like thallium content in the blood. The value of the correlation coefficient \( (r) \) between the thallium content in the feed and the contents of the eggs was 0.9931 \( (p \leq 0.01). \)

Haematological Parameters

The mean number of erythrocytes in the blood in the control group was 2.55 mln/l, and in the experimental groups ranged from 2.48-2.60 mln/l. The differences between the groups were not statistically significant. The level of hemoglobin in the control group was 7.77 mmol/l, while the values in groups II, III, and IV were slightly higher – group II: 8.01 mmol/l; group III: 8.07 mmol/l; group IV: 8.34 mmol/l. However, the differences in the hemoglobin values between the groups were not statistically significant. The mean number of leukocytes in the control group was 31.18 thousand/l. In group II, the mean number of...
white blood cells was 32.01 thousand/l and in group III 32.83 thousand/l. The highest level of leukocytes was observed in the group IV, in which the white blood cell number was 38.73 thousand/l. The difference between the leukocytes in group IV, compared to the control group, was statistically highly significant. The hematocrit index in the control group was 24.54%. In the second group the index was 25.73% and in group III 25.92%. In the last group (IV) the hematocrit value was 27.56%, which was higher than groups I (p ≤ 0.01) and II (p ≤ 0.05).

Biochemical Parameters

The biochemical tests of blood have shown that the level of GOT in the control group (I) was 174.67 U/l, whereas in the experimental groups this enzymatic activity was slightly higher, with values between 193.73-200.05 U/l. However, mentioned differences were not statistically significant. The level GPT in all groups was similar and ranged from 19.96 (group I) up to 21.51 U/l (group II). The creatinine concentration in the control group was 26.15 mmol/l. In the intoxicated groups (II, III, and IV) the value was almost twice higher than the control group (p ≤ 0.01). The concentration levels of creatinine in experimental groups II-IV were not statistically different and were noticed in a range of 47.40-55.29 mmol/l. Blood glucose levels in groups treated with thallium (group II, III, IV) were lower than the control group (p ≤ 0.01 and p ≤ 0.05). This value in the control group was 12.22 mmol/l, while the level of glucose in the intoxicated groups was in the range of 11.03 (group II) up to 11.44 mmol/l (group III). There were no statistically significant differences between the experimental groups. Analyzing the level of the cations sodium, potassium, and chloride anions, no statistically significant differences between groups were observed. The content of Na+ peaked the values between 153.71-156.29 mmol/l, K+ from 4.35-4.49 mmol/l, and the level of Cl- ranged 122.37-125.39 mmol/l.

Discussion

The study showed that 8 weeks of hen intoxication by the subtoxical thallium doses caused its accumulation in the tissues, internal organs, and eggs of these birds, and the concentration was correlated with thallium content in the feed. The experimental thallium concentrations in the feed (2.6-16 mg/kg) corresponded to the mean content of this element in the food products from the industrial areas [18-20]. The experimental concentrations correspond to a daily dose of -0.14, 0.38, and 0.74 mg/kg/day in groups II, III, and IV, respectively, and resulted in subtoxical doses that can make an impact on them only after prolonged exposure and accumulation in the body of this element. Unfortunately, the human studies in the field are well known, but the complemented animal experiments are rare. The lethal dose LD₅₀ for children is only 8-15 mg/kg, 10-15 mg/kg in adult humans [20, 21], while for laboratory animals such as mice, rats, guinea pigs, and rabbits it’s 15-50 mg/kg [4], and 50 mg/kg for wild ducks [22]. Death and serious toxic effects may also occur at lower doses [23-28].

In our study the level of thallium in the blood clearly depends on the level of dose as well as exposure time, which was the result of constant animal feeding, which was poisoned by thallium and its absorption from the gastrointestinal tracts with the higher impact on the animals’ body, rather than a single dose of poison. The fast increase in the level of thallium in eggs and blood in the first few weeks of the experiment and a high correlation between thallium content in the feed and the concentration of thallium in tissues and between the levels of thallium in various tissues, indicates the easy absorption of this element by the gastrointestinal tract and its rapid distribution in the body. The distribution of this element from the blood into the tissues occurs quickly. The biological half-life of thallium in the blood of rats is less than 5 minutes [20]. The first symptoms of thallium poisoning can be observed at least in 1-2 days of the poison administration, which allows for early detection and avoidance of serious neurological symptoms [23, 17, 29-31]. The analysis of the distribution of thallium in tissues of hens in our study clearly shows that the greatest concentration of this element occurred in the bones and kidneys, muscles and livers. Similar results were noticed by Ueberschär et al. [32], who analyzed bioconcentrations of thallium in laying hens and broiler chicken tissues intoxicated at a dose of 2 up to 40 mg per 1 kg of feed. The authors demonstrated that this element was laid down mainly in the kidneys, the bones, muscles, and livers, similar to our study. It should be noticed that the concentration levels in the tissues of broilers was higher than in hens. The high concentrations of thallium are observed in the kidneys, which is a result of the renal filtration of intracellular accumu-

<table>
<thead>
<tr>
<th>Group</th>
<th>Thallium content in blood [mg/l]</th>
<th>Thallium content in tissues [mg/kg]</th>
<th>Thallium content in eggs [mg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>breast muscles</td>
<td>thigh muscles</td>
<td>liver</td>
</tr>
<tr>
<td>I</td>
<td>0.34</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>II</td>
<td>59.66</td>
<td>0.76</td>
<td>0.74</td>
</tr>
<tr>
<td>III</td>
<td>157.66</td>
<td>1.72</td>
<td>1.76</td>
</tr>
<tr>
<td>IV</td>
<td>248.87</td>
<td>2.70</td>
<td>2.95</td>
</tr>
</tbody>
</table>
mulation of thallium [24]. It also has been reported that bovines living in industrial areas have a thallium concentration in the kidneys at a level of 24 mg/kg, in the liver 2.3 mg/kg, and in the muscles of 0.4 mg/kg [33]. Munch et al. [34] during the analysis of the thallium content in fox tissues showed the highest concentration in the liver in these animals is 64 mg/kg and then in the intestines (55 mg/kg) and kidneys (34 mg/kg). The literature also reports [19] that the concentration of thallium in sheep in contaminated regions was 29 mg/kg in kidneys and 15.8 mg/kg in livers, and in pigs 1.2 mg/kg in livers, 1.2 mg/kg in kidneys, and 0.6 mg/kg in muscles. Similarly, other authors [13, 17, 26, 27, 35] have demonstrated that human beings poisoned by thallium, the highest concentration was observed in the kidneys and bones, stomach and intestine, spleen, liver, muscle, lung, and brain. In studies on the toxicity of thallium in dogs the highest concentrations of thallium were noticed in the blood cardiac muscle and kidney [29, 30]. Moreover, the studies of the thallium distribution in various skeletal muscles in rabbits and rats [36] at 1.5 and 24 hours after thallium administration showed significant differences between the accumulation of this element in the individual muscles. However, in our study there were no significant differences between the thallium concentration breast muscle and the thigh muscle. During acute poisoning of the wild ducks [22] it was also shown that the highest concentrations of thallium were observed in the kidneys, liver, blood, muscle, heart, and lungs, but this element is not shown in the bones. It should be noticed that this research was made with acute poisoning as a result of the sudden death of birds.

In our study it was shown that during the chronic thallium intoxication in laying hens there was a strong accumulation of this element in the eggs. The thallium content in the eggs also depends on the dose and duration of exposure. In the available literature [2, 3] there is only one report that in the region close to a cement plant in Lengerich (Germany), Tl concentrations in whole eggs were 1.26 mg/kg fresh matter, 0.394 mg/kg of fresh matter in egg white and yolk, and 4.94 mg/kg of fresh matter in eggshells. Thallium is an element that also passes through other barriers in the mammal’s bodies, such as brain and the glands [17, 27, 35]. It was also pointed out by Lapointe and Couture (2010) during the case study on the fatted minnows that the results of dietary metal accumulation may be different according to environmental factors or animal species and its conditions [37]. In the clinical trials there were no signs of acute toxicity of thallium, which could be characteristic and well described in the literature as the best syndromes of Tl intoxication. [35, 38, 39]. Even if acute toxicity appears, there is a strong correlation with other non-typical factors as gastroenteritis, bradycardia, dermatitis, or CNS pathology in humans [8, 27, 21, 35]. Moreover, in many countries there are no threshold limits of thallium in the foodstuffs for millions of consumers [39]. In animal studies, it was mentioned that sheep died without any outward signs of disorders, the rabbits and horses were reported to lose their hair because of heavy thallium contamination. Nevertheless, there is little knowledge about the small doses of this element in animals (II).

In the experimental animals (laying hens) the subclinical changes in haematological and biochemical tests were observed. These changes include an increased number of white blood cells, creatinine, and hematocrit, as well as the decrease in blood glucose levels. In studies on the effects of chronic thallium intoxication in laboratory animals the authors pointed to the similar changes in biochemical and hematological blood parameters [40, 41]. The increase in serum creatinine suggests that the high levels of thallium in kidneys contribute to their dysfunction. Impaired renal filtration processes will decrease thallium elimination from the body and lead to further accumulation.

The results of this study indicate that the applied thallium dose did not cause clinical signs of poisoning, but led to accumulation of this element in the body and disorders in the homeostasis of the hens’ bodies.

Conclusions

Chronic thallium intoxication with subtoxical doses causes bioaccumulation of this element in the body of laying hens, proportionally to the duration of exposure and the dose which was used. Moreover, it was proven that the highest concentrations of thallium are presented firstly in the bones and afterward in the kidneys, muscles, livers, and at last in the blood. The concentration of thallium in the eggs was also proportional to the dose of this element in the feed and the time of the experiment. Finally, it should be pointed that chronic thallium intoxication with the use of subtoxical doses in hens did not induce typical and clinical symptoms of thalliosis, and the only subclinical signs of the thallium presence in the organism was increased hematocrit, white blood cell numbers, and creatinine levels and a decrease in blood glucose levels.

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