

Chronic exposure to cobalt compounds – an *in vivo* study

Research Article

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Abstract: An *in vivo* experimental model for testing the effects of long-term chronic treatment with cobalt(II) compounds – cobalt chloride (CoCl₂) and cobalt-EDTA (Co-EDTA) on mice at different stages of development was optimized. Pregnant mice and their progeny were treated with daily doses of 75 or 125 mg kg⁻¹ body weight until postnatal day 90. The compounds were dissolved in regular tap water. Mice were sacrificed on days 18, 25, 30, 45, 60 and 90 after birth, which correspond to different stages of their development. Altered organ weight indices (calculated as a ratio of organ weight to body weight) of spleen, liver and kidneys, were found depending on the type of compound used, dose, duration of treatment, and the age of the animals. The results also showed significant accumulation of cobalt ions in blood plasma, spleen, liver and kidneys of the exposed mice. More Co(II) was measured in the organs of the immature mice (day 18, 25 and 30 pnd) indicating that they were more sensitive to treatment.

Keywords: Cobalt chloride • Cobalt EDTA • Mice • Blood plasma • Spleen • Liver • Kidney

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1. Introduction

Cobalt(II) is a widely used substance that can be found in nutritional supplements, preservatives, drinks, cosmetics, medical devices, and is used as a therapeutic agent for treating various diseases. The exposure to cobalt (Co) from industry and surgical implants requires thorough studies for biological effects of the metal ions. For the general population, diet (meat, vegetables, drinking water) is the main source of Co. Studies on long-term exposure in laboratory animals to the metal ions show that they accumulate in the kidney, liver, spleen, heart, stomach, intestines, muscle, brain and testes [1]. The concentration of Co(II) is also increased in whole blood, serum and urine [2,3]. Co treatment is shown to improve tissue adaptation to hypoxia, enhance physical endurance

and performance, and ameliorates mountain sickness [3]. Its salts affect the body weight of patients and experimental animals, but the mechanism remains to be elucidated [4]. Chronic exposure also causes allergic contact dermatitis and diseases of the upper respiratory tract [5]. The wide use of cobalt alloys in medical devices requires full elucidation of its biological role in cells, tissues and organs after long-term exposure [6,7]. Data show that cobalt is transferred from food into mother's milk [8,9]. Young animals (rats and guinea pigs) have a 3- to 15-fold greater absorption than adult animals (aged 200 days or more) [10]. Although found widely in the environment, diet is the main source of cobalt(II) for humans and animals. The average daily intake of cobalt ranges from 5-45 µg with relatively high concentrations of the metal occurring in fish and in vegetables [11].

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The aim of the present study was to determine the effects of *in vivo* chronic exposure to cobalt(II) compounds – cobalt chloride (CoCl_2) and cobalt-EDTA (Co-EDTA) on the spleen, liver, and kidneys of mice from different age groups. Plasma cobalt content was studied as well.

2. Experimental Procedures

2.1 Animal model

Pregnant ICR mice were subjected to daily doses of 75 and/or 125 mg kg^{-1} body weight cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) and/or Co-EDTA 2–3 days before they gave birth to their progeny. The compounds were dissolved and delivered in drinking tap water. Our previous experience has shown that each mouse drinks approximately 8 ml water/day, therefore the required dose was dissolved in 8 ml per mouse per day. After birth, we continued to treat the mothers with the same dose because cobalt is transferred into the milk and thus the newborn mice were exposed to the metal ions. When the newborn mice were 25 days old they were separated into individual cages to ensure that all experimental animals obtained the required daily dose and treatment continued until they were 90 days old. The mice were weighed weekly and the Co concentration in the water was adjusted to correspond with body weight. In our previous experiments we found no significant gender differences, either in body weight or in haematological parameters, and the experimental groups consisted of equal number of male and female mice. Animals were fed a standard diet and had access to the food *ad libitum* with strong control of the feeding regime. The mice were maintained in the Institute's animal breeding facility at $23 \pm 2^\circ\text{C}$ and 12:12 h light/dark cycles, in individual standard hard-bottom polypropylene cages. The animals were sacrificed by decapitation after etherization on days 18, 25, 30, 45, 60, and 90, which correspond to different stages of development. Each group consisted of 7 mice for CoCl_2 and 5 for Co-EDTA experimental design. Whole blood samples were obtained, centrifuged, and the plasma was stored at -20°C until needed for further analysis. The control group consisted of age-matched mice drinking regular tap water. The study was approved by the Ethics Committee of the Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences.

2.2 Morphological studies

Spleen, liver and kidneys were excised and weighed. Spleen index (SI), liver index (LI) and kidney index (KI) were calculated as a ratio of organ weight to body weight.

2.3 Analysis of cobalt concentration in blood plasma

Cobalt concentration in blood plasma was determined by electrothermal atomic absorption spectrometry (ET-AAS) on Zeeman Perkin Elmer 3030, HGA 600.

2.4 Analysis of cobalt concentration in spleen, liver and kidney

Cobalt (II) bioaccumulation in the spleen was determined after nitric acid wet digestion flame atomic absorption spectrometry (FAAS) and using Perkin Elmer AAnalyst 400, flame:air-acetylene.

2.5 Statistical analysis

Results are presented as mean value $\pm$ SD. Statistical significance between the experimental groups was determined using two-tailed Student's *t*-test for independent samples. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

Chronic treatment with cobalt(II) compounds led to accumulation of the metal's ions in the blood plasma of the experimental animals (Figures 1,2). The highest Co(II) content was measured in day 18 mice exposed to CoCl_2 treatment. Accumulation of cobalt(II) in blood plasma induced hemoglobin changes, thus affecting hematopoiesis [12].

Chronic treatment with cobalt(II) compounds also induced significant accumulation of the metal ions in the spleen, liver and kidneys of the exposed mice.

Long-term exposure to CoCl_2 and Co-EDTA altered spleen index (SI) of treated mice (Table 1). The results indicate that both compounds affect differently immature and mature mice. Since the ratio of splenic weight to body weight remains fairly constant regardless of age [13], changes in this parameter may be used as an indicator of exposure. Since it is also a storage site for iron, erythrocytes and platelets [13], alterations in its functions will affect iron metabolism, blood cell production, and possibly other functions as well.

The spleen of mice exposed to Co-EDTA accumulated more Co(II) compared to those that obtained CoCl_2 . This tendency was observed for both daily doses used in the experiment – 75 mg kg^{-1} and 125 mg kg^{-1} . The same tendency was found for liver and kidneys as well (Figures 3, 4).

Accumulation of cobalt(II) in the spleen affects extramedullary hematopoiesis, as previously described [14].

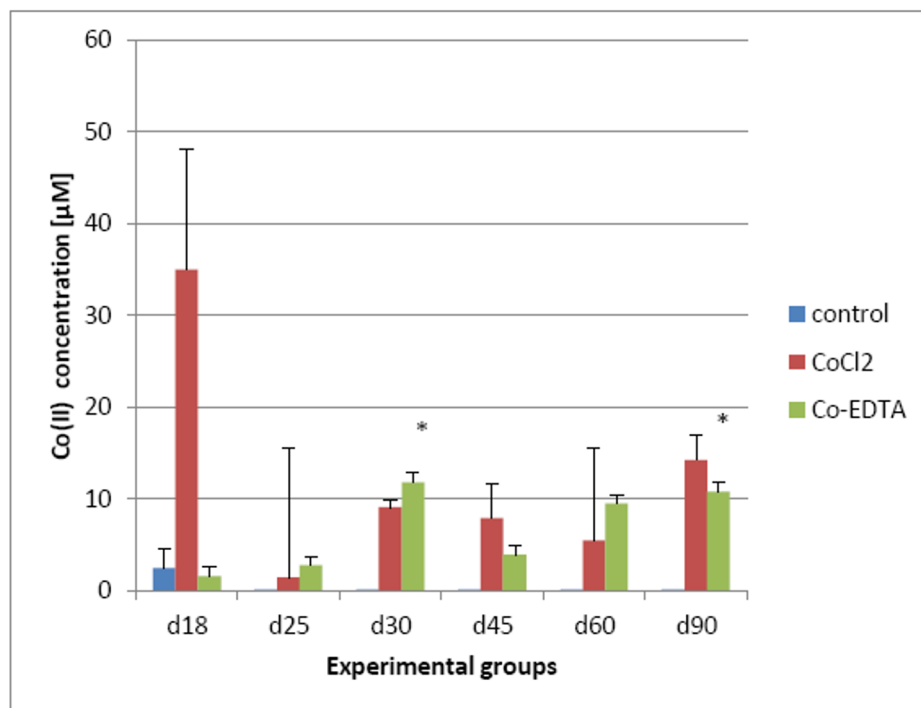


Figure 1. Cobalt(II) content in blood plasma of mice treated with low daily dose of 75 mg kg⁻¹ CoCl₂ (n=7) and/or Co-EDTA (n=5). Data are presented as mean±SD. Single asterisk (*) represents statistical difference ($p < 0.05$).

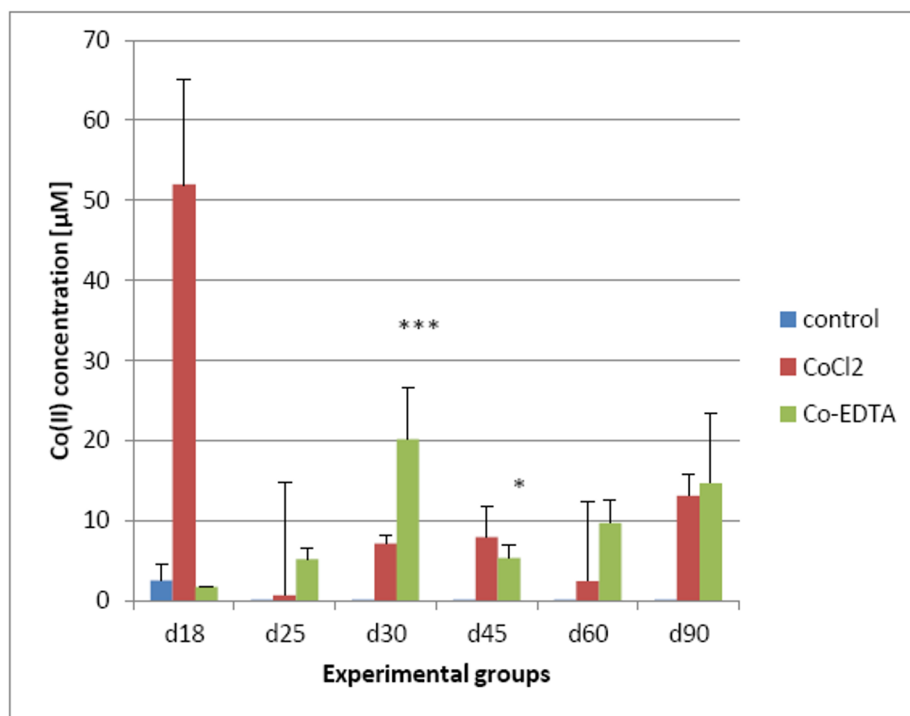


Figure 2. Cobalt(II) content in blood plasma of mice treated with low daily dose of 125 mg kg⁻¹ CoCl₂ (n=7) and/or Co-EDTA (n=5). Data are presented as mean±SD. Single asterisk (*) represents statistical difference ($p < 0.05$), triple asterisk (***) represents ($p < 0.0001$).

Experimental group	Spleen index (%)	
	CoCl ₂	Co-EDTA
d18 control		
75 mg/kg	-	42.55↓ ***
125 mg/kg	9↓	25.53↓
d25 control		
75 mg/kg	18 ↑	6.53↑
125 mg/kg	-	3.9↑
d30 control		
75 mg/kg	7.7↑***	92.3↑*
125 mg/kg	61.52↓***	33.3↑
d45 control		
75 mg/kg	11.9↑	57.14↑***
125 mg/kg	26.2↑	61.9↓***
d60 control		
75 mg/kg	42.5↓***	10↑
125 mg/kg	-	15↑
d90 control		
75 mg/kg	52.7↓*	10.9↑
125 mg/kg	20↓*	29.1↓

Table 1. Changes in spleen index after chronic treatment with CoCl₂ (n=7) and Co-EDTA (n=5). Single asterisk (*) represents statistical difference ($p < 0.05$), triple asterisk (***) represents ($p < 0.0001$).

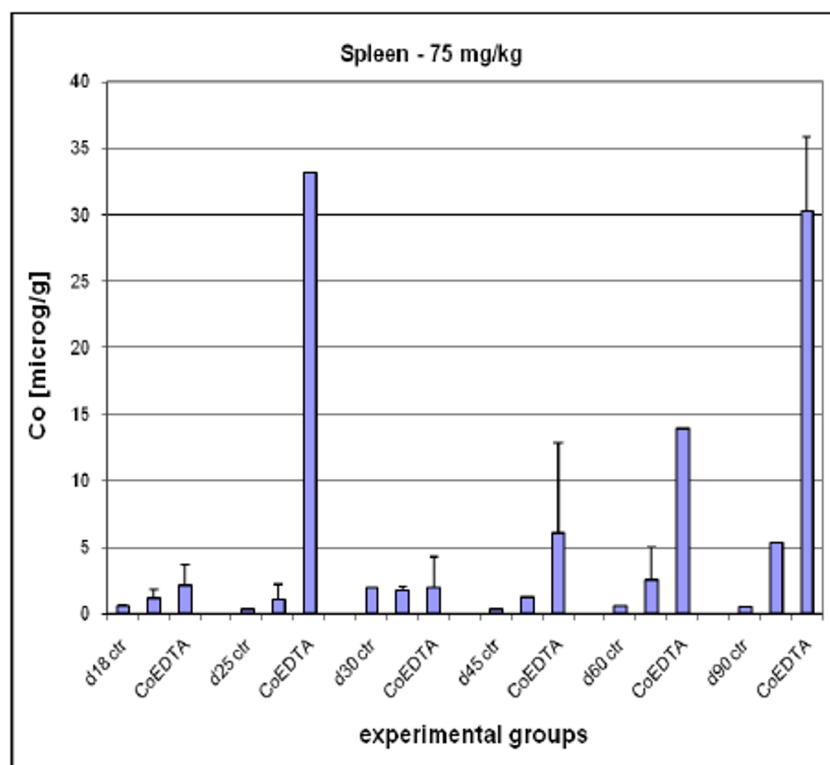


Figure 3. Cobalt(II) content in the spleen of mice treated with low daily dose of 75 mg kg⁻¹ CoCl₂ (n=7) or Co-EDTA (n=5). Data are presented as mean ± SD.

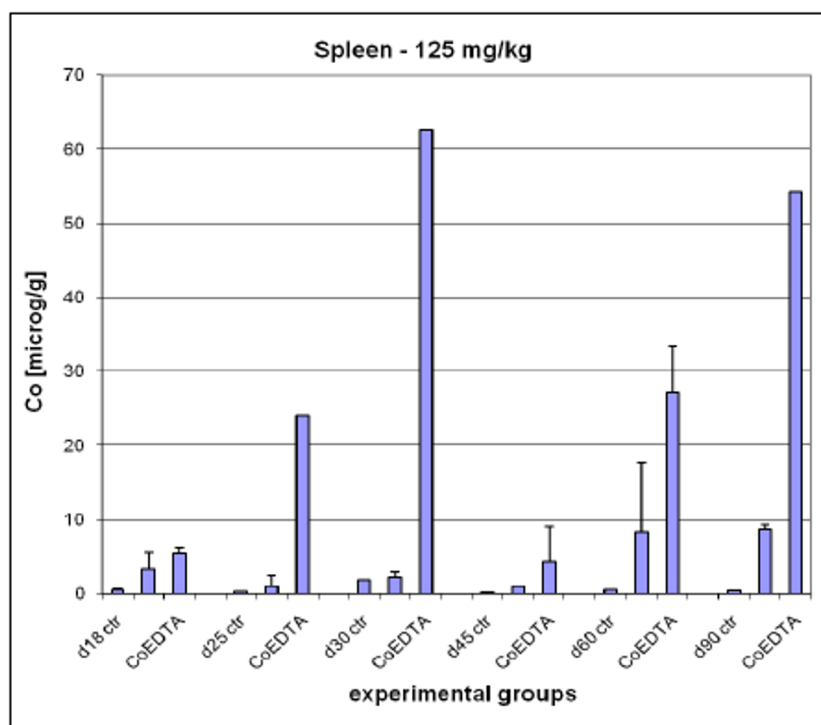


Figure 4. Cobalt(II) content in the spleen of mice treated with high daily dose of 125 mg kg⁻¹ CoCl₂ (n=7) or Co-EDTA (n=5). Data are presented as mean ± SD.

Chronic treatment with Co(II) compounds altered liver index (LI) as well (Table 2). Exposure to CoCl₂ induced a decrease in LI, while Co-EDTA had the opposite effect.

This could be correlated with the higher Co(II) content in the liver of mice treated with Co-EDTA (Figures 5, 6). Our results for CoCl₂ are in agreement with those of Garoui *et al.* for decreased liver weight in day 14 rats [15]. The increased LI after Co-EDTA exposure is in agreement with Cupertino *et al.* [16] showing increased liver somatic index in rats after chronic treatment with cadmium. The results indicate that the biological effect of cobalt depends on the type of compound it forms. Additionally, the ligand should also be considered.

More cobalt was accumulated in the organs of immature mice compared to the mature animals. This indicates that the immature are more sensitive to treatment, possibly due to the lack of certain enzyme systems. A similar effect was observed for the kidneys (Figures 7, 8).

The Kidney index (KI) on the other hand was significantly increased in day 30 mice after treatment with CoCl₂ (Table 3). The compound had little or no effect on KI of mature animals. The effect of Co-EDTA was diverse and no clear tendency was observed.

When compared with the changes of LI it seems that when KI is increased, LI is either decreased or no change is found. A possible relationship between the two requires further exploration. The increased KI could be due to inflammation or stimulated angiogenesis as observed by Tanaka *et al.* [17]. The authors also demonstrate the renoprotective effect of CoCl₂ in rats.

Exposure to Co(II) was reported to have caused the syndrome of “beer drinker’s cardiomyopathy” in Quebec City, Canada, characterized by pericardial effusion, and congestive heart failure [11]. In our study changes in the heart (somatic index and histology) were not studied so far and are planned as future work.

4. Conclusion

Chronic treatment with cobalt(II) compounds led to significant accumulation of metal ions in the blood plasma, spleen, liver and kidneys of exposed mice. Increased concentrations of Co(II) were measured in the liver and kidneys of the immature mice treated with Co-EDTA. The results indicate that Co(II) accumulation depends on the type of compound cobalt forms, the duration of exposure, as well as on the age of the exposed animals.

Experimental group	Liver index (%)	
	CoCl ₂	Co-EDTA
d18 control		
75 mg/kg	-	10.9↓*
125 mg/kg	13↓***	-
d25 control		
75 mg/kg	12.5↑	50↑
125 mg/kg	9.4↑	59.4↑
d30 control		
75 mg/kg	13↓**	9.3↑
125 mg/kg	22↓	5.5↑
d45 control		
75 mg/kg	11.7↓	10↑
125 mg/kg	18.3↓*	20↑
d60 control		
75 mg/kg	1.9↓	3.7↑
125 mg/kg	9.3↓	16.7↑***
d90 control		
75 mg/kg	21.5↓	9.2↓
125 mg/kg	15.4↓	20↓

Table 2. Changes in liver index after chronic treatment with CoCl₂ (n=7) and Co-EDTA (n=5). Single asterisk (*) represents statistical difference ($p < 0.05$), double asterisk (**) represents ($p < 0.001$), triple asterisk (***) represents ($p < 0.0001$).

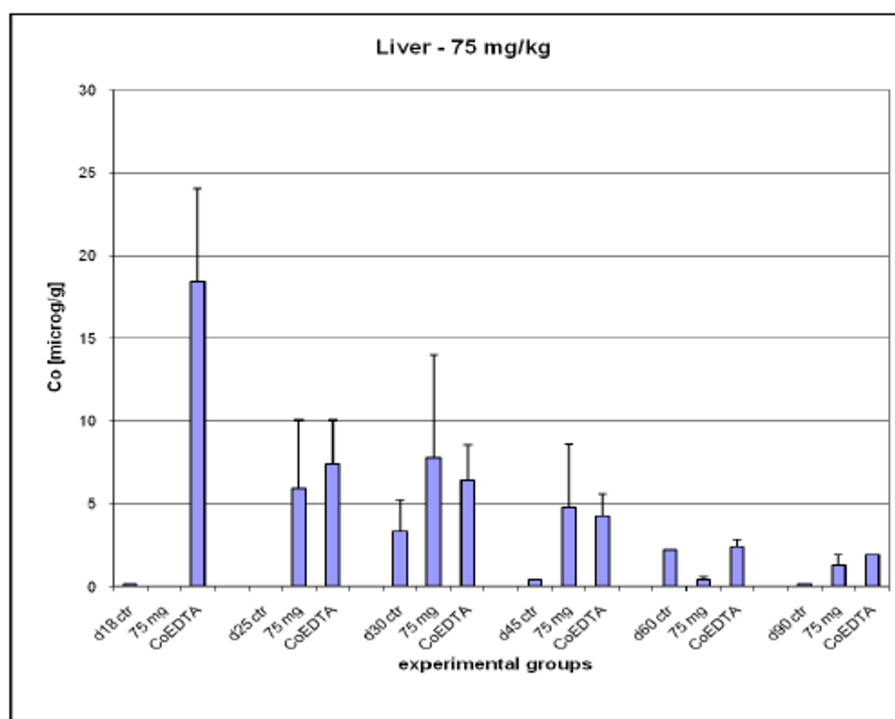


Figure 5. Cobalt(II) content in the liver of mice treated with low daily dose of 75 mg kg⁻¹ CoCl₂ (n=7) and/or Co-EDTA (n=5). Data are presented as mean ± SD.

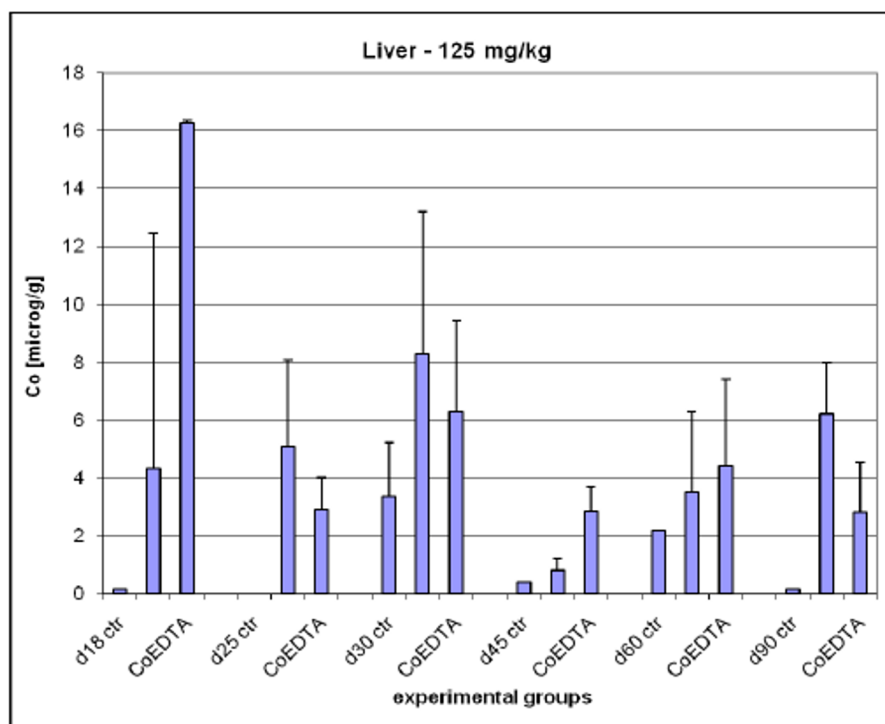


Figure 6. Cobalt(II) content in the liver of mice treated with low daily dose of 125 mg/kg CoCl_2 ($n=7$) and/or Co-EDTA ($n=5$). Data are presented as mean $\pm$ SD.

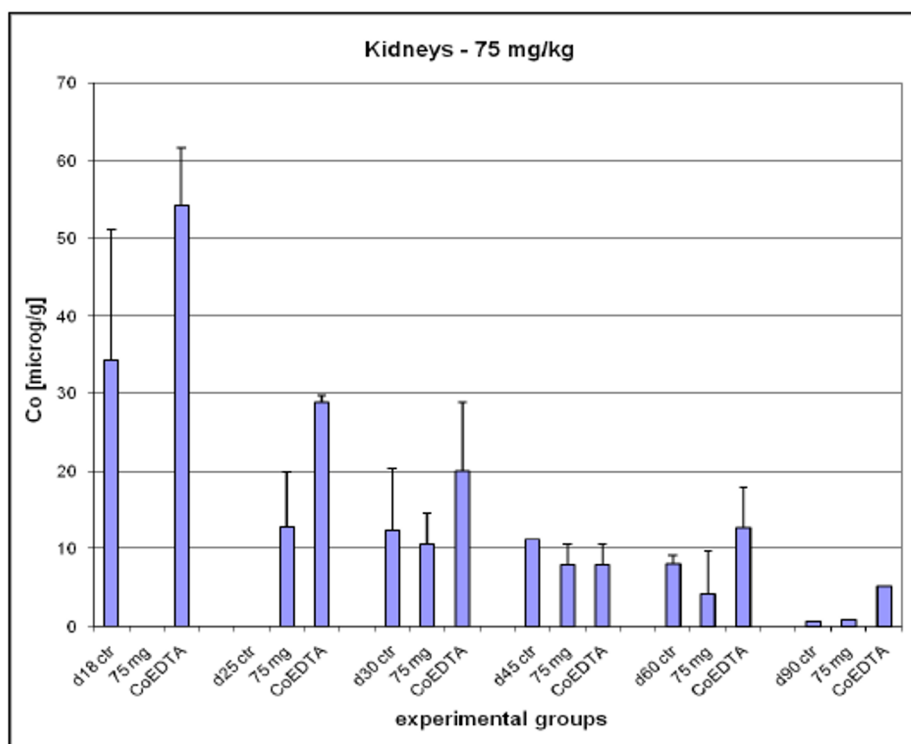


Figure 7. Cobalt(II) content in the kidneys of mice treated with low daily dose of 75 mg kg^{-1} CoCl_2 ($n=7$) and/or Co-EDTA ($n=5$). Data are presented as mean $\pm$ SD.

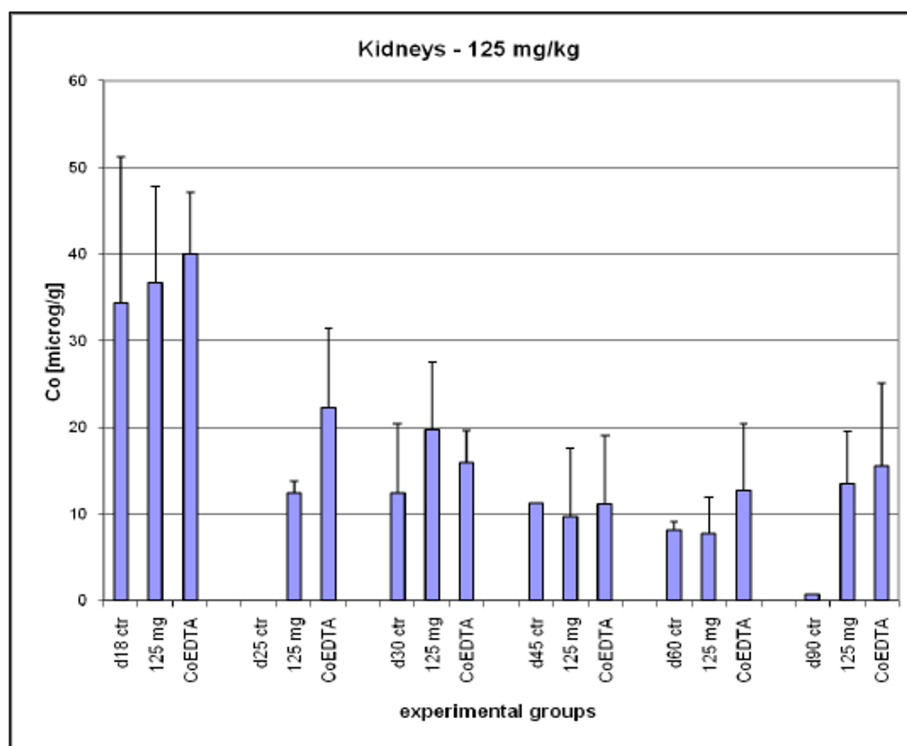


Figure 8. Cobalt(II) content in the kidneys of mice treated with low daily dose of 125 mg kg⁻¹ CoCl₂ (n=7) and/or Co-EDTA (n=5). Data are presented as mean±SD.

Experimental group	Kidney index (%)	
	CoCl ₂	Co-EDTA
d18 control		
75 mg/kg	-	6.7↑
125 mg/kg	6.7↑	-
d25 control		
75 mg/kg	-	-
125 mg/kg	-	-
d30 control		
75 mg/kg	14.3↑	7.1↓
125 mg/kg	28.6↑***	7.1↓***
d45 control		
75 mg/kg	-	12.5↑***
125 mg/kg	-	6.3↑
d60 control		
75 mg/kg	-	5.9↓
125 mg/kg	-	-
d90 control		
75 mg/kg	7.7↑	30.8↑
125 mg/kg	7.7↓	15.4↑*

Table 3. Changes in kidney index after chronic treatment with CoCl₂ (n=7) and Co-EDTA (n=5). Single asterisk (*) represents statistical difference ($p < 0.05$), triple asterisk (***) represents ($p < 0.0001$).

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