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Cumulative rate and distribution of Cd and Pb in the organs of adult male Wistar rats during oral exposure

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ABSTRACT

The degree of accumulation of Cd and Pb in the brains, spleens, lungs, hearts, livers and kidneys of adult Wistar rats was compared both for separate (Cd or Pb) and combined (Cd + Pb) oral exposure. In addition, the metals were administered either with liquids or with solid feed. Rats were exposed to low doses of metals (7 mg Cd and 50 mg Pb/kg feed or L of distilled water) over 6 or 12 weeks. In total the organs of rats accumulated about 0.3–0.5% Cd and 0.4–0.6% Pb supplied with food or drink. The presented studies demonstrated that the distribution of Cd and Pb in the organs is affected by: the type of exposure (separate or combined), the source of metals (feed or drinks) and the duration of exposure. It was found that simultaneous exposure to low doses of Cd and Pb supplied with food is much more hazardous than exposure to such metals supplied with water.

1. Introduction

Cadmium (Cd; half-life 5–30 years) and lead (Pb; half-life 30 days) are toxic metals that are rapidly absorbed from the alimentary tract because they easily penetrate through the biological barriers in the organism and are characterised by high accumulability in soft tissues (EFSA, 2009, 2010; Tong et al., 2000). The cumulative increase in the content of toxic metals in animal and human tissues is connected with their passage to the higher level of the food chain (Castelli et al., 2005). Respective organs are exposed to a different degree of the toxic effects of harmful metals. These xenobiotics are mainly accumulated in the liver, kidneys and bones, leading to their damage (Dai et al., 2013; Trzcinka-Ochocka et al., 2010). Living organisms show very poor ability to excrete toxic metals. Small amounts of Cd may be excreted from the body in urine and/or via bile (Zalups and Ahmad, 2003).

The gastrointestinal tract is chronically exposed to usually small amounts of toxic metals. The extent of absorption of Cd supplied orally is approximately 1–8% (Ohta et al., 2000), while that of Pb is 10–50% (Järup, 2003). The extent of absorption of Cd and Pb depends, among other factors, on the age of animals, their gender, metabolic rate, diet components, and the dose of metal (Andersen et al., 2004; Horiguchi et al., 2004; Maciak et al., 2011; Ohta et al., 2000; Satarug et al., 2001). Available literature contains little information about the percentage of Cd and Pb absorption by respective internal organs

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Table 1 – Experimental design.

Groups	Weeks of exposure	n	Treatment		Matrix	Access to food and water
			Cd[a]	Pb[b]		
D-Cd	6	6	7 mg/L	–	Distilled water	Free
	12	6	7 mg/L	–	Distilled water	Free
D-Pb	6	6	–	50 mg/L	Distilled water	Free
	12	6	–	50 mg/L	Distilled water	Free
D-Cd + Pb	6	6	7 mg/L	50 mg/L	Distilled water	Free
	12	6	7 mg/L	50 mg/L	Distilled water	Free
F-Cd	6	6	7 mg/kg	–	Feed	Free
	12	6	7 mg/kg	–	Feed	Free
F-Pb	6	6	–	50 mg/kg	Feed	Free
	12	6	–	50 mg/kg	Feed	Free
F-Cd + Pb	6	6	7 mg/kg	50 mg/kg	Feed	Free
	12	6	7 mg/kg	50 mg/kg	Feed	Free

[a] As $CdCl_2$.
[b] As $(CH_3COO)_2Pb$.

compared to the amount consumed orally, both in animals and humans. However, numerous studies present information on Cd and Pb accumulating in tissues but they do not account for the intake level of these metals.

The study aimed to compare the degree of absorption of Cd and Pb in the brains, spleens, lungs, hearts, livers and kidneys of adult Wistar rats during oral exposure to low doses of metals. Three factors were examined: (1) type of exposure – separate (Cd or Pb) or combined (Cd + Pb), (2) source of metals – drinks or feed and (3) duration of exposure – 6 or 12 weeks.

2. Materials and methods

2.1. Animals

The Local Ethics Committee for Human and Animal Experiments at the University of Life Sciences in Lublin (Poland) approved the experiment involving adult (aged 84 days, mean body weight 342.8 ± 12.4 g) male Wistar rats. The rats were housed individually in a polypropylene cage in an air-conditioned room (temperature 21–23 °C, humidity 45–55%, 12-h light-dark cycle). Rats were acclimatised to laboratory conditions for 1 week.

2.2. Treatments

For the purposes of the experiment it was assumed that rats would be exposed to low doses of metals, lower than environmental exposure, the value of which was determined during experiments with rats by Kaczmarek-Wdowiak et al. (2004). In this study the mean level of Cd was about 2.5 mg/kg body weight/week whereas the mean level of Pb was 18–21 mg/kg body weigh/week. Rats, split into 6 experimental groups (D-Cd, D-Pb, D-Cd + Pb, F-Cd, F-Pb, F-Cd + Pb), received Cd and Pb with feed or drinks over 6 or 12 weeks, in doses of respectively 7 mg Cd (as $CdCl_2$) and/or 50 mg Pb (as $(CH_3COO)_2Pb$) per 1 kg or per 1 L, over 6 or 12 weeks, as presented in Table 1. Two methods of exposure were utilised: separate exposure (Cd or Pb) and combined exposure (Cd + Pb). Feed and drinking fluids intake were measured daily, body weights were measured weekly. Rats were sacrificed at the end of 6th ($n = 6$) and 12th ($n = 6$) weeks. Rats were starved for 24 h and then disposed of by breaking their spinal cord after previous treatment with CO_2. After bloodying the rats, their brains, spleens, lungs, hearts, livers and kidneys were prepared as a whole. The organs were weighed, and each put into a separate plastic bag and frozen at a temperature of −20 °C.

2.3. Cd and Pb analysis

A portion of the sample of tissues (~3 g) was subjected to mineralisation. Dry ashing was conducted in a muffle furnace at a temperature of 450 °C for 12 h. The resulting ashes were transferred to test tubes and dissolved in 10 mL of nitric acid solution (1 M HNO_3). The content of Cd and Pb was determined by the atomic absorption spectrophotometry with hydride generator in a Varian Spectr AA 880 apparatus, as shown in Table 2. Detailed methods for determining Cd and Pb were presented in another work (Winiarska-Mieczan, 2013).

2.4. Chemicals

Cadmium chloride $CdCl_2$ and lead acetate $(CH_3COO)_2Pb$ were purchased from POCH S.A. (Poland). All chemicals used were of

Table 2 – Measurement parameters for the determination of Pb and Cd.

	Pb	Cd
Wave length (nm)	217.0	228.8
Lamp current (mA)	10	4
Spectral band pass (nm)	1	0.5
LOD (mg/kg)	0.011	0.001
LOQ (mg/kg)	0.03	0.004
Pure gas	Argon	Argon
Background correction	Zeeman	Zeeman
Mean recovery rate	95%	96%
The deviation of duplicate measurement	4.6%	5.5%
Quality control		
Blank sample	1 M HNO_3	1 M HNO_3
Certified reference material	185R (bovine liver)	185R (bovine liver)