A new index for heavy metals in biological monitoring

MARIKO MOCHIZUKI¹, MAKOTO MORI², RYO HONDO³ and FUKIKO UEDA³)*

¹) Department of Applied Science, School of Veterinary Nursing and Technology, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, 1-7-1 Kyounan, Musashino, Tokyo 180-8602, Japan,
²) Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan,
³) Laboratory of Veterinary Public Health, School of Veterinary Medicine, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, 1-7-1 Kyounan, Musashino, Tokyo 180-8602, Japan

*Corresponding author: Fukiko Ueda at above address.
E-Mail: fueda@nvlu.ac.jp

Abstract: - This study was designed to verify the usefulness of the cadmium (Cd) standard regression line (CSRL) in animals uncontaminated with lead (Pb), and to develop a new index for Pb if the CSRL could not be used. Although a significant regression line for Pb was obtained using a total of 69 data points cited in 23 previously published references, four data points were eliminated by the statistical examination (min-Pb line), and the min-Pb line was significantly different from the CSRL. The data from animals to which Pb was administered experimentally and from wildlife obviously polluted with Pb diverged from the min-Pb line. Furthermore, contents of other heavy metals, namely chromium, copper, manganese, zinc, arsenic, cobalt and selenium, in normal animals, cited from previously published references, also fell on the min-Pb line, although the data for Tl (thallium) fell on the CSRL. We named the min-Pb line the ‘Mochizuki–Ueda line’ because this line may become a widely used index for animals uncontaminated by multiple metals.

Key-Words: - biological monitoring, index, wildlife, experimental animal, lead, cadmium, heavy metals

1 Introduction

Diseases caused by exposure to environmental contamination with toxic heavy metals, such as Itai-Itai disease caused by cadmium (Cd) and Minamata disease caused by mercury (Hg) in Japan, have become worldwide problems. Therefore, we need to monitor environmental pollutants constantly. Many researchers have used biological monitoring of wildlife as a method of environmental monitoring. We have also reported the contamination of wild birds by several elements, including Cd [12], thallium (Tl) [13], chromium (Cr) [16], molybdenum (Mo) [16], and vanadium (V) [17]. However, there is often a discrepancy between the concentration of pollutants in the environment and the biological effects observed. This is due to the lack of epidemiological data on the sex, age and feeding habits of the animals, and on the food-chains that involve the animals and the differences between species. Furthermore, it is difficult to distinguish uncontaminated wildlife from contaminated. We have developed a new biological index (the cadmium standard regression line; CSRL [14]) for species uncontaminated with Cd, using measurements on liver and kidney samples, by integration of data from previously published references. The CSRL is derived from 62 species uncontaminated with Cd, and it has a fixed range of Cd contents in their organs, because we considered the differences in sex, age and species to be regulated by an equal probability ellipse (Cd equal probability ellipse; CEPE, [19]).

Metallothionein (MT), a metal binding protein, is known to yield as a combination with zinc (Zn) in animals; the Zn ions may be substituted by several metals such as Cd, mercury (Hg) and lead (Pb). The Cd–MT, Hg–MT and Pb–MT compounds are excreted from the body [5]. This suggests that data on these metals should fall on the CSRL or near to the CSRL. Therefore, the first aim of this study was to verify whether the CSRL can be used as an index for animals uncontaminated with Pb. If the CSRL cannot be used for Pb, the second aim was to develop a new index for Pb.
The aim of this study was to verify whether these formulas can be used as the indexes for animals uncontaminated with Pb. We used the computer software packages Excel 2003 (Microsoft), Excel statistical add-in software (Microsoft), Lotus 2001 (INSO) and JMP (SAS) for all statistical analyses.

3 Problem Solution

3.1 Review of previous reports on Pb, and construction of the regression line
In this review we selected references in which particular types of contamination of animals were not described. Twenty-three previously published references that reported Pb contents in various animals were employed for the review. These references reported 69 data points from a total of 40 species: land birds and waterfowl, 9 species; seabirds, 5 species; terrestrial mammals, 11 species; marine mammals, 4 species, and freshwater fish, 11 species. When the 69 data points were plotted on a graph with the Pb content in the liver on the abscissa and that in the kidney on the ordinate, a good correlation was obtained, as shown in Fig. 1. The formula of this regression line (the Pb line) was:

\[
\log(Y) = 0.973 \log(X) + 0.012, \quad R^2 = 0.861, \quad P < 0.01 \quad (4)
\]

Figure 2 shows the Pb line and the 95% equal probability ellipse for formula (4); four points on the
Pb line fell outside the 95% equal probability ellipse, although there were no descriptions of Pb contamination in the relevant references. The slope and the absolute term in formula (4) were slightly changed after elimination of the four points. The formula of this minimum-Pb (min-Pb) line was:

\[
\log(Y) = 0.988 \log(X) - 0.015, \quad R^2 = 0.861, \quad P < 0.01 \quad (5)
\]

Here, the \(a_5\) and \(b_5\) of the equal probability ellipse \(X^2/(a_3)^2 + Y^2/(b_3)^2 = 1\) for formula (5) were: \(a_3 = 0.390, b_3 = 2.022, \) and the \(\tan2\theta_5 = -14.74\)

However, there was no significant difference between formulas (4) and (5).

### 3.2 Comparison of Pb line with the CSRL

Figure 3 shows the relationship between the min-Pb line and the CSRL with the 95% equal probability ellipses. The ranges were seen to be different, and therefore we compared statistically formulas (4) and (5) for the Pb and min-Pb lines with formulas (2) and (3) for the CSRL and min-CSRL, respectively. Table 1

<table>
<thead>
<tr>
<th>difference</th>
<th>slope</th>
<th>absolute term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistics value</td>
<td>1.001137</td>
<td>15.83378</td>
</tr>
<tr>
<td>degree of freedom (d.f.)</td>
<td>155</td>
<td>156</td>
</tr>
<tr>
<td>t ratio (0.025)</td>
<td>1.975387</td>
<td>1.975387</td>
</tr>
<tr>
<td>P value</td>
<td>0.318321</td>
<td>3.33E-34</td>
</tr>
</tbody>
</table>

| Statistics value | 131.6025 | 2 |
| d.f. (1) | 155 |
| d.f. (2) | 3.054385 |
| P-value | 3.92E-34 |

judgment | equal | different |
shows the results of the statistical test for the two regression lines: the min-Pb line (5) with 65 data points and the min-CSRL (3) with 94 data points. Two absolute terms were significantly different, while the slopes were the same. Although similar results were obtained with formulas (2), (3), (4) and (5), the P value from the comparison of formulas (3) and (5) was the lowest. These facts suggest that the Pb line is different from the CSRL, and that we should use the min-Pb line and min-CSRL to distinguish Pb contamination and Cd contamination. However, there was still no evidence that the Pb line and the ellipse can be used to identify Pb-contaminated animals. Thus, we attempted to validate it by use of data from previously published references.

3.3 Validation of min-Pb line using data from experimental animals and wildlife

The Pb line was compared with the data in previously published references for animals to which Pb was experimentally administered and for wildlife obviously polluted with Pb (Fig. 4).

**Mouse:** Xie et al. [21] administered 400 mg Pb^{2+}/ml of Pb acetate solution daily as drinking water to a total of 10 mice for 10 days. The reported data were added to a graph of the min-Pb line with the 95% equal probability ellipse. The Cd content in their control mice (see the symbol * in the figure) was a low value, although the data from their control was also outlier. The Pb contents from the mice to which Pb was administered were high and diverged significantly from the min-Pb line, and were of course outside the 95% ellipse.

**Condor and turkey:** Pb poisoning due to Pb shot has been reported in several species of wildlife [8], and the experimental administration of Pb shot has been also reported in several animals. Californian condors were dosed with Pb shot for 50 days by Pattee et al. [18]. Turkeys were dosed with Pb shot by Carpenter et al. [3] and had a survival time of 143–211 days. Data from both the condors and turkeys given Pb indicated very high contents, and diverged completely from the min-Pb line, as shown in the figure.

**Pb-contaminated wildlife:** Beyer et al. [2] reported high levels of Pb contamination in wild songbirds living in a mining district. Kim and Koo [9] suspected acute exposure to Pb in two heron species in Korea. High contents of Pb have also been reported in waterbuck in Kenya [7] and in flamingo contaminated by Pb shot [1]. The data obtained from these references also diverged from the min-Pb line.
3.4 Relationship between the Pb line and other elements

Given that we need also to monitor the contamination of the environment by other toxic heavy metals, several elements, including Cr, Tl, Zn, copper (Cu), manganese (Mn), arsenic (As), cobalt (Co), selenium (Se), and nickel (Ni), were compared with the min-Pb line and min-CSRL (Fig. 5). These data were obtained from previously published references for several species of wildlife \([4,11,21]\), and there were no descriptions of contamination by these heavy metals in the references. The data for Co, As, Cr and Mn were within the 95% equal probability ellipse of the min-Pb line, Cu and Se were slightly out of the ellipse, and Zn diverged completely from the ellipse. However, these seven metals agreed with the min-Pb line, at least in some respects. The data for Tl agreed completely with the min-CSRL, but judgment was difficult for a similarly low value of Ni.

It would be best if we could generate a single index for the monitoring of environmental contamination by many toxic heavy metals. The present results showed that data from eight metals, including Pb, fell on the min-Pb line and those from two metals, Tl and Cd, fell on the min-CSRL. Thus, we have named the min-Pb line the ‘Mochizuki–Ueda line’, because this line may become a widely used index for animals uncontaminated by multiple metals, although this line is different from the min-CSRL. However, problems still remain, because data for Cu, Se, Zn, Tl and Ni lie outside the 95% equal probability ellipses of both lines. The ranges of the 95% equal probability ellipses for these metals may be different from both the Mochizuki–Ueda line and the min-CSRL. These two indexes should therefore be verified by more studies to increase their usefulness in biological monitoring.

4 Conclusion
In this study, it was determined whether CSRL, which we have been advocating as index for animals uncontaminated with Cd, could be used to monitor environmental pollution by Pb, which combines with MT in the body in a similar way to Cd. A significant correlation between measurements from kidney and liver was obtained in the Pb contents from uncontaminated animals in previously published references, although four data points were eliminated.
by the statistical examination (min-Pb line). It became clear that the min-Pb line was significantly different from the CSRL. Data from experimental animals and wildlife contaminated by Pb diverged significantly from the min-Pb line. Furthermore, the contents of Cr, Cu, Mn, Zn, As, Co and Se from uncontaminated animals also fell on the min-Pb line, although those from animals uncontaminated with Tl fell on the CSRL. We have named the min-Pb line the ‘Mochizuki–Ueda line’ because this line may be widely used as an index for animals uncontaminated by multiple metals.

References: