行政院國家科學委員會補助專題研究計畫成果報告

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計畫類別：□x **個別型計畫**　　□**整合型計畫**

計畫編號：NSC 89－2320－B－002－037

執行期間：88 **年** 8 **月** 1 **日至** 89 **年** 7 **月** 31 **日**

計畫主持人：　黃耀輝

執行單位：　國立台灣大學公共衛生學院
職業醫學與工業衛生研究所

中　華　民　國　89 **年** 10 **月** 31 **日**

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摘要

本計劃研究目的為分析鉛蓄電池廠鉛塵之物化特性，並評估其相對生物攝取率。動物實驗部分以九十隻雄性 Sprague-Dawley **大鼠進行餵食含不同鉛粉塵飼料之觀察，餵食劑量分別設定** 5 **及** 10 mg/KgBW/day**，觀察期為** 2 **至** 6 **週。研究結果顯示，陰極鉛粉和陽極極板整理粉塵的組成成分為鉛與二氧化鉛，其他類別的粉塵則僅含元素態鉛。鉛粉原料的粒徑全部小於** 80um**，眾數大小為** 11~12 um**。** 66%~75%**的極板整理粉塵之粒徑小於** 250 um**，而僅** 27%**的極板裁切粉塵小** 250um**。陰極鉛粉原料、陰極極板整理粉塵及極板裁切粉塵的重量組成中，幾乎全是鉛及其化合物。另外，一般而言實驗動物的血鉛值會隨攝食之鉛粉塵粒徑減小而增加較快。並且，高劑量組的實驗顯示，在第二週即可達到體內穩定血中鉛值；而低劑量組則要在第四週才可達到。在相同暴露劑量的狀況下，各種不同鉛粉塵來源的生物攝取率並不同，最低的是由** 106um~250um **粒徑範圍的陰極極板粉塵所觀察到的** 30.9%**生物攝取率，最高的是由眾數粒徑為** 12um **的陰極鉛粉原料所觀察到的** 245.1%**。本計劃研究結果可提供進一步鉛蓄電池廠鉛暴露風險評估的重要參數，特別是有關於不經意狀況下食入前粉塵或由上呼吸到排除轉移到腸胃道到吸收的鉛塵暴露。**

關鍵詞：鉛、生物攝取率、動物研究、鉛蓄電池廠、粉塵。

ABSTRACT

This study was conducted to characterize the physicochemical properties of the lead powder/dust from a lead battery plant, and estimate their relative bioavailability. Ninety male Sprague-Dawley rats were fed with lead-dosed mixtures at the target doses of 5 and 10 mg/Kg BW/day, respectively, during the specified study periods. Results shows that lead powder for anode and the lead dust from the cathode plate manipulating process consisted of both lead element and lead oxide while the other types of lead powder or dust were found composed of lead element only. Also, it is shown that the lead powder was the smallest in particle size with a mode of 11~12 um, while 66%~75% of lead dust from plate manipulating process and 27% of that from plate cutting process were composed of particle size less 250 um. Lead powder for anode plate, lead dust from the anode electrode plate manipulating process, and from the plate cutting process were mostly composed of lead and/or its compounds, in terms of weight percentage. Generally speaking, the blood lead levels increased inversely with the particle size of the lead powder and/or dust, and reached the plateau level around the 4^{th} week for the low dose group and about the 2^{nd} week for the high dose group. At the same lead exposure dose, the bioavailability of lead uptake vary from 30.9% for the lead dust from anode plate manipulating process with size range of 106um~250um to 245.1% of lead powder for anode plate with size mode of 12 um, depending on the lead source and the time period of blood collection. The observations

in the present study provide important parameters for the improved risk assessment of exposure to lead dust in the lead battery plant, especially for inadvertent ingestion and/or ingestion of coarse particles transferred from the naso-pharyngeal area to the gastro-intestinal tract.

Keywords: Lead, Bioavailability, Animal Study, Lead Battery Plant, Dust.

INTRODUCTION

Delineation of the lead pathway of workplace to house indicates that lead is probably transported into the houses on the clothes, shoes, hair, skin and, in some case, motor vehicles of the workers. Meanwhile, lead contamination on mouth and hand has also been indicated in a lead battery plant that parenteral intake from hand and mouth contamination is an important cause of lead absorption in lead-exposed workers. In order to delineate the contribution to the blood lead level from ingestion, this study was conducted with a living animal model to estimate the relative bioavailability of lead dust in the lead battery plant, and analyze the physicochemical characteristics of the dust lead. Results of this study would provide a basis for improved exposure and risk assessment.

MATERIAL AND METHOD

The test materials were lead powder and dust samples obtained from a lead battery plant, including lead powder for cathode plate, lead powder for anode plate, lead dust from cathode plate manipulating process, lead dust from anode plate manipulating process, and lead dust of electrode-plate cutting. The first part of this study was the determination of the physicochemical characteristics, i.e., the surface characteristic, weight distribution by size range, size distribution, components, and the lead percentage in weight of the study powder and dust samples. Ninety male Sprague-Dawley rats (nine/dose group; 6-7 weeks of age at initiation of dosing) were supplied by the National Laboratory Animal Breeding and Research Center, National Science Council (Taipei, Taiwan, R.O.C.). Animal were individually housed in standard polycarbonate metabolic cages for rats (Nalgene). All animals were provided deionized water *ad libitum* by glass bottle reservoirs fitted with stainless steel sipper tubes and fed twice per day. Every morning, depending on the stages of the study period, the study animals were fed various amount of purified diet AIN-76 complete meal *ad libitum*, from 20 to 22 g, while lead-dosed complete meal with exact lead content were served late afternoon at a specified limited amount of one fiftieth of the rat's body weight to make sure these meals would be finished during the night hours. The dosed feed group animals had access to lead mixed feed till the terminations of each observation period, i.e., 14, 28, 42 days, respectively. The low and high target dose levels were selected for each study lead-dose feed mixture at 5 and 10 mg Pb/kg/day, respectively. For the reference group, the animals were fed a purified diet AIN-76® into which the appropriate amount of lead acetate were added to replace the lead dose as mentioned above. Dosed feed concentrations, stability, and homogeneity were verified during the study. During the in-life phase, clinical observations, food consumption, and body weight determinations were recorded for further analysis. Daily cage checks were made twice for morbidity and mortality, and daily clinical observations were made for any possible signs for toxicity. At the end of the specified observation periods, three rats were etherized and sacrificed for blood collection by drawing from the femoral vein. Blood specimens were stored and refrigerated until removed for analysis.

The study lead dust/powder samples were first sieved with ASTM standard sieve with U.S.A. No. 140 and 60 mesh, equivalent to 106 um and 250 um in opening size (VWR Scientific Inc. subsidiary of Univar). Pre-sieved lead dust and powder samples were examined with the scanning electron

microscope (SEM, Hitachi-S 800) to examine the surface characteristics of the study particulates. Lead dust/powder was further analyzed for the particulate size distribution with laser light diffraction (Coutler LS 230). Furthermore, the components of study lead samples were characterized with the X-ray diffraction (XRD, M03XRF) to determine the primary compositions inside the study samples. Lead content determination for the dust/powder samples was performed on the graphite atomic absorption spectrometer (GAAS, Perkin Eemer® 5100), so was for the dosed feed mixtures.

Bioavailability was calculated by comparing the blood lead concentrations of animal fed with the study lead powder/dust samples to those with lead acetate. For example, a feed group was treated with lead dose of X_0, compared to those reference group treated with lead acetate, and their corresponding blood levels were presented as a dose-response function of X_0, i.e., $f_{treat}(X_0)$, $f_{ref}(X_0)$, respectively. Given the background blood concentration of the study animal was c ug/dl, and the $f_{treat}(X_0)$, $f_{treat}(X_0)$ for a given time t were b ug/dl and c ug/dl, respectively, then the relative bioavailability for time t could be expressed as follows:

Relative Bioavailability (t)
= [(b-c)/(a-c)]x100%

According the experimental design, the relative bioavailability were calculated at the 15^{th}, 29^{th}, and 43^{rd} days following the sacrifice and blood collection.

RESULTS AND DISCUSSION:

Physicochemical characteristics of lead dust/powder

1. Description of the particulate surface:
For particulates of lead powder for either cathode or anode plate, most are spherical, smoother and more homogeneous in size and shape compared to the lead dust obtained from the electrode plate manipulating process, both cathode and anode. For the latter ones, most of them are in irregular shape, pile-up, and with rough surface, and varying in size. For the lead dust from the plate cutting process, the particulates share the similar characteristics of lead dust from the electrode plate manipulating process. Figure 1 shows the topography of the particulate surface of the lead dust from the anode plate manipulating process as an example for demonstration.

2. Size distribution, weight percentage and lead contents:

Size distribution, weight percentage and lead contents of particulates were summarized in Table 1. It is shown that all the lead powder was less than 106 um in size, with a mode of 11~12 um, while 66%~75% of lead dust from plate manipulating process was composed of particle size less 250 um, and only 27% of that from plate cutting process was less than 250um. On the other hand, lead powder for anode plate, lead dust from the anode electrode plate manipulating process, and from the plate cutting process were mostly composed of lead and/or its compounds, percentage in weight ranging from 96.0% to 102.1%, while slightly lower lead contents were found in the lead dust from cathode plate manipulating process and the lead powder for cathode, varying from 80.0% to 90.5%.

3. Physicochemical properties of lead powder and dust.

Table 2 presents the physicochemical properties of the study lead powder and dust. Lead powder for anode and the lead dust from the cathode plate manipulating process consisted of both lead element (Pb) and lead oxide (PbO_2) while the other types of lead powder or dust were found composed of lead element only. Also shown in Table 2 is the crystal system, crystal parameter, and color of these study lead powder and dust.

4. Body weight and lead dose uptake in

animal study

Table 2 presents the average body weights of the study animals by week, increasing from around 200 g at the beginning to about 450 g for the last group sacrificed at the end of the 6th week. And, according to the experimental design, the average dose of lead uptake of the study animals were set at 5 or 10 mg Pb/kgBW/day, i.e., target doses. However, due to the varying lead content percentage of each type of study powder/dust, and the variation of exact amount added to the feed mixture, the actual lead doses applied to the study animals might somewhat deviate from the preset target dose. Nevertheless, most of the average actual lead dose were controlled within 10% of the target lead dose.

5. Bioavailability

Table 4 shows the blood lead levels of the study animals at the specified time frame of sacrifice. The highest blood lead levels were found around 110~120 ug/dl among the rat group fed with high dose lead powder for anode plate, particle size mode of 12 um, while the lowest blood lead levels of 30's ug/dl were observed among the rats treated with low level lead dust from the anode plate manipulating process. Generally speaking, the blood lead levels increased inversely with the particle size of the lead powder and/or dust. And, the rat groups fed with low lead-dosed feed mixtures had blood lead levels reached plateau of blood lead level around the 4th week while that of the high lead-dosed group usually arrived at the blood lead plateau levels at the end of the 2nd week. Compared to the reference group dosed with lead acetate, the relative bioavailability was calculated and shown in Table 4. For exposure to the same type of lead dust or powder, the rats fed with high lead-dosed feed mixtures showed elevated blood lead levels up to around 110% compared to those fed with low lead-dosed, depending the types of lead sources and the time period of exposure, i.e., the specific time of sacrifice for blood collection. On the other hand, since these study lead powder/dust were composed of high percentage of lead contents and the dose used to feed the study animal were calculated based on the actual lead amount in the feeding, we may simply estimate the size effect of lead-containing particle on the blood lead levels, without paying too much attention on whether it is powder or dust in the present study. Therefore, at the same lead exposure dose, the bioavailability of lead uptake vary from 30.9% for the lead dust from anode plate manipulating process with size range of 106um~250um to 245.1% of lead powder for anode plate with size mode of 12 um, depending on the lead source and the time period of blood collection.

SELF ASSESSMENT:

The present study has been conducted well following the original study design except the solubility study of the lead dust and/or powder under the simulated gastro-intestinal environment. Since the current study is an animal exposure study, the study results reflected the outcome resulting from complicated metabolism process after ingesting lead-dosed feed mixtures. Therefore, it is believed, with the results of this *in vivo* study, the omission of the part of solubility study won't alleviate the virtue of the present study. Meanwhile, the observations in the present study has successfully achieve the preset goals of characterizing the lead sources encountered in the lead battery plant and assessing the relative effects of dose, types of sources, and particle size on the lead powder/dust's contribution to blood lead levels. Prior to the present study, there is rare information on physicochemical and bioavailability regarding *in situ* lead source of the lead battery plant. The observations of this study, therefore, provide important parameters for the following risk assessment of exposure to lead dust in the lead battery plant, especially for inadvertent ingestion and/or ingestion of coarse particles transferred from the naso-pharyngeal area to the gastro-intestinal tract.

A draft of article based on this study is now being prepared and expected to be submitted

an international journal for publication. Meanwhile, the study also fostered two master theses which are listed in the reference and contain more details regarding the present study.

REFERENCE

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Table 1 . Lead source, particle size and weight distribution, and lead content percentage of particulates.

Lead sources	Particle size range, μm	Weight proportion by size range, %	Particle size mode, μm	Lead content in particulate, %
Powder, Anode	<106	100	12	96.0
Powder, Cathode	<106	100	14	90.5
Dust, Plate Manipulating Process, Anode	>250	43.0	—	—
	106-250	23.2	140	99.6
	<106	33.8	61	98.8
Dust, Plate Manipulating Process, Cathode	>250	47.5	—	—
	106-250	28.3	169	83.6
	<106	24.3	4	80.0
Cutting Plate	>250	72.7	—	96.8
	106-250	11.0	154	102.1
	<106	16.3	80	102.0

Table 2. Physicochemical properties of lead powder and dust.

Lead sources	Elements & compounds	Crystal system	Crystal parameters	Color
Power, Anode	Pb PbO_2 *	cubic tetragonal	a=4.9506Å **a=4.9525**Å **c=3.3863** Å	sliver gray black
Power, Cathode	Pb	cubic	a=4.9506 Å **a=4.9525** Å **c=3.3863** Å	sliver gray black
Dust, Plate Manipulating Process, Anode	Pb	cubic	a=4.9506 Å	sliver gray
Dust, Plate Manipulating Process, Cathode	Pb PbO_2 *	cubic tetragonal	a=4.9506Å **a=4.9525**Å **c=4.3863** Å	sliver gray black
Cutting Plate	Pb	cubic	a=4.9506Å	sliver gray

* Plattnerite

Table 3. Body weights and exposed lead doses for the study animals.

Lead Sources	Lead Dose, mg Pb/Kg BW/day		Average Body Weight at the i^{th} day, Mean±SD (g)						
	Target	Actual	0	7	14	21	28	35	43*
Lead Acetate	5	5.96	224.3 (10.4)	269.2 (16.4)	310.9 (20.8)	346.8 (25.8)	381.0 (31.9)	390.0 (30.0)	418.0 (36.2)
	10	10.77	205.3 (15.0)	253.1 (20.3)	290.7 (29.0)	328.0 (49.9)	367.5 (58.4)	417.3 (40.5)	449.0 (44.4)
Plate Manipulating, Anode, <106 um.	5	5.92	216.3 (11.6)	270.4 (16.5)	317.7 (18.5)	355.3 (15.1)	389.3 (14.8)	418.7 (22.1)	452.3 (18.0)
	10	9.33	239.7 (20.4)	285.6 (20.7)	336.2 (19.4)	370.7 (27.2)	406.3 (32.4)	418.3 (16.8)	442.7 (25.5)
Plate Manipulating, Anode, 106~250 um.	5	4.62	209.7 (5.5)	258.3 (3.5)	321.0 (3.6)	391.7 (8.5)	434.7 (13.1)	430.0 (24.6)	454.3 (30.0)
	10	9.19	169.3 (2.5)	230.7 (5.1)	294.7 (6.43)	311.3 (11.2)	344.7 (21.1)	438.7 (8.0)	472.3 (2.1)
Plate Cutting, <106 um	10	9.13	171.0 (9.6)	229.3 (15.3)	294.3 (18.8)	343.3 (10.7)	371.3 (33.2)	416.3 (9.3)	445.7 (13.1)
Lead Powder, Anode	5	4.52	196.0 (6.1)	256.7 (7.4)	314.0 (8.2)	365.0 (19.9)	408.3 (21.7)	421.3 (26.8)	443.7 (45.6)
	10	9.08	215.3 (12.3)	268.3 (28.4)	321.7 (29.5)	362.3 (4.7)	409.0 (7.5)	438.3 (26.3)	471.7 (26.9)
Control	0	None	211.7 (28.0)	274.0 (35.9)	340.0 (36.7)	387.3 (18.9)	422.7 (21.6)	456.0 (15.6)	484.7 (26.3)

Table 4. Blood lead levels and bioavailability of study animals by batch of sacrifice.*,**

Dose Group	Lead Sources	Actual Pb Dose, mg Pb/ KgBW/day		i^{th} day of the study period		
				15th	29th	43rd
Low	Lead Acetate	5.96	Blood Lead Level, ug/dl	39.8 (3.6)	62.2 (8.8)	57.4 (10.5)
			Bioavailability	100%	100%	100%
	Plate Manipu-lat-ing, Anode, <106 um	5.92	Blood Lead Level, ug/dl	31.3 (6.4)	30.9 (1.7)	34.6 (5.7)
			Bioavailability	69.4%	37.2%	49.5%
	Plate Manipu-lating, Anode, 106~250 um	4.62	Blood Lead Level, ug/dl	37.5 (7.7)	47.1 (4.4)	54.2 (9.1)
			Bioavailability	118.2%	89.8%	119.8%
	Lead Powder, Anode	4.52	Blood Lead Level, ug/dl	57.8 (5.9)	63.0 (12.4)	75.0 (9.2)
			Bioavailability	218.8%	134.0%	183.5%
High	Lead Acetate	10.77	Blood Lead Level, ug/dl	67.8 (13.9)	58.8 (9.7)	64.4 (9.7))
			Bioavailability	100%	100%	100%
	Plate Manipu-lating, Anode, <106 um	9.33	Blood Lead Level, ug/dl	36.7 (9.0)	49.3 (10.4)	61.0 (9.7)
			Bioavailability	50.6%	91.8%	108.3%
	Plate Manipu-lating, Anode, 106~250 um	9.19	Blood Lead Level, ug/dl	36.2 (12.8)	58.1 (15.4)	77.3 (8.4)
			Bioavailability	50.2%	115.4%	146.3%
	Plate cutting, ???um	9.13	Blood Lead Level, ug/dl	65.9 (6.7)	88.0 (16.5)	92.7 (22.3)
			Bioavailability	113.9%	192.4%	182.3%
	Lead Powder, Anode, ???um	9.08	Blood Lead Level, ug/dl	110.0 (14.5)	103.5 (5.4)	120.0 (26.6)
			Bioavailability	209.1%	233.1%	245.7%

* Each batch was composed of 3 rats.

** Bioavailability was calculated by comparing the blood lead levels of rats ingesting lead-dosed feed mixtures to those of the control group which were fed with mixtures containing lead acetate, after deducting the background blood lead level of 12.5ug/dl obtained from the measurement of rats at the beginning of the present study without lead powder/dust exposure. Meanwhile, adjustments were made for the unequivalent lead dose between actual doses between the lead-dust/powder treated group and the lead acetate control group.

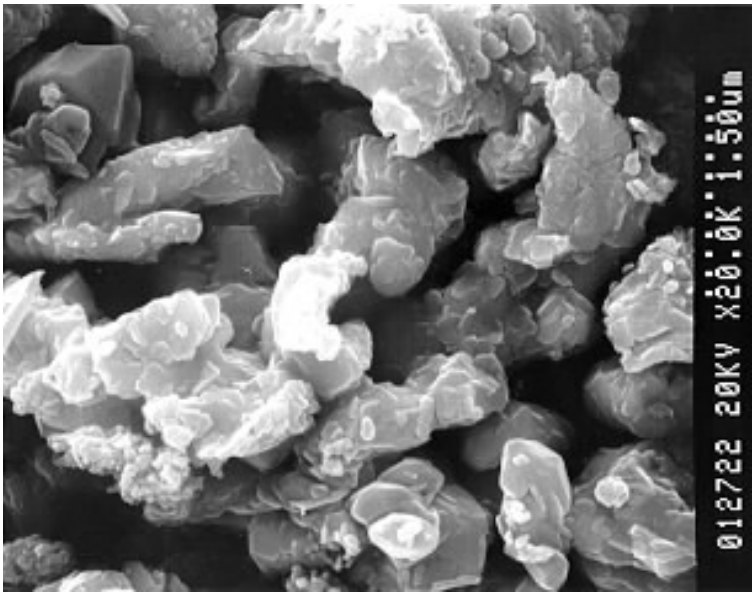

Figure 1. Topography of the particulate surface of the lead dust from the anode plate manipulating process, under the scanning electron microscope (SEM).