LD_{50} DETERMINATION OF ZINC PHOSPHIDE TOXICITY FOR HOUSE MICE AND ALBINO LABORATORY MICE

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ABSTRACT: Results showed that wild house mice were more susceptible to zinc phosphide than an albino strain. The LD_{50} and 95% confidence limits for combined sexes with wild house mice (Mus musculus) were 32.68 ± 3.58 mg/kg, but 53.34 ± 2.64 for the albino mice. The regression equations between the probits of mortalities and the logs of doses are Y=10.38x-10.72 for wild mice and Y=6.78x-6.71 for albino mice. Both the individual and sexual variations among albino mice in their susceptibility to zinc phosphide were greater than those of wild mice. The results suggest that attempts to extrapolate toxicity values from albino mice to wild mice may prove misleading.

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INTRODUCTION

Of the acute rodenticides, zinc phosphide (Zn_3P_2) is most commonly used (Wang and Pan 1981) and has a relatively long history of use for commensal and field rodents (Schoof 1970). More and more it is becoming a standard to compare with newly developed rodenticides (EPPO 1975). The house mouse (Mus musculus) is one of the most widely distributed commensal rodents and is of significant economic importance. Albino mice are often used as a convenient substitute for wild house mice in the first stages of experiments with new rodenticides.

METHODS

An LD_{50} was determined for both wild and albino laboratory mice according to the procedures described by Sun (1963), as explained below. The Swiss-Webster strain of albino mice was obtained from Simonsen Laboratories, Inc., Gilroy, California. The wild mice were from our breeding colony at the Ecology Institute facility, University of California, Davis. The mice were individually caged, acclimated to the laboratory, and observed for health verification for 7 days prior to testing.

The zinc phosphide (94% purity) was the product of Industrial Chemicals Division, Hooker Chemical Corporation, a subsidiary of Occidental Petroleum Corporation, New York. The zinc phosphide powder was weighed and mixed with an appropriate amount of corn oil to establish the necessary concentrations for intubating mice at 0.2 ml/10-g body weight. The zinc phosphide was kept in suspension with a magnetic stirrer until administered orally with a syringe equipped with a 5-cm ball-tipped, 18-gauge curved gavaging needle.

A pretest was conducted to establish the range of toxicity so that the proper dose levels could be established for LD_{50} determinations. Each pretest used groups of 5 male and 5 female mice. Three dose levels (10, 40, and 160 mg/kg) of zinc phosphide were used in the pretesting. Based on the pretest results, final dosages were established with each group comprised of 10 animals. Food was removed from each animal's cage about 4 hours before the rodenticide suspension was intubated. Each animal was observed daily for 5 days following dosing. The albino mice were sexually mature animals, ranging in weight from 17 to 30 grams and the mature wild mice ranged from 13 to 25 grams.

The LD_{50} determinations were based on dose levels that increased by a geometrical progression. Five or six dose levels were ultimately required for establishing the LD_{50}s. Calculations were based on the following formulas (Sun 1963):

A. LD_{50}, STANDARD ERROR (SE_{50}) AND CONFIDENCE LIMIT (d_{50})

The LD_{50} is calculated accordingly:

$$\log LD_{50} = Xm - i\left(\sum p - \frac{3-Pm-Pn}{4}\right)$$

where log LD_{50}, Xm, and "i" are the logarithm of the LD_{50}, of the highest dose tested and the common ratio, respectively (take the larger dose as numerator in the ratio). "Pm" is the mortality of the highest dose group while "Pn" represents that of the lowest. Pm should not be less than 80% and Pn should not exceed 20%. When Pm=1.0 and Pn=0.0, the following simpler formula can be used:

$$\log LD_{50} = Xm - i\left(\sum p - 0.5\right)$$

The standard error of LD_{50} can be calculated as follows:

$$SE = i \times \sqrt{\frac{\Sigma p - \Sigma p^2}{n - 1}}$$

In this formula Σp^2 is the sum of squares of mortalities. Letter "n" stands for the number of animals per dose level tested, and the number should be the same (10 animals) for all groups. The 95% confidence limit of LD_{50} is:

$$d50 = LD_{50} \pm 4.5 \times LD_{50} \times SE_{50}$$

B. REGRESSION EQUATION

$$Y = 5 + b\,(x - \log LD_{50})$$

Where "Y" presents the probit of a mortality, "x" is the logarithm of the corresponding dose, "b" is the regression coefficient, and

$$b = \frac{Yh - Yl}{i(N-H)}$$

Yh is the average probit of the half of the groups that had the higher tested doses, and YL is the average probit of the half of the groups that had the lower tested doses. N is the number of the groups tested. H is half the number of the groups tested. If the N is an even number, H=N/2; if N is an odd number, H=(N-1)/2. The standard error of regression coefficient "b" is

$$Sb = \frac{1}{i} \times \sqrt{\frac{12 \times (Y - Ye)^2}{(N-2)\,(N-1)\,N\,(N+1)}}$$

where "Y" is the probit values corresponding to each dose obtained from the test. "Ye" is the probit values corresponding to each dose obtained from the regression equation as it applies to the test. Y is actually the measured value of probit and Ye the estimated value of probit from the regression equation.

$\Sigma\,(Y-Ye)^2$ is the sum of squares of differences between Y and Ye.

C. LDk, SEk AND dk (95% CONFIDENCE LIMIT)

In addition to LD_{50}, SE_{50}, and d_{50}, we can work out the LDk, SEk and dk where "k" represents any value from 0 to 100 we want to know using the following formulas:

$$\log LDk = \log LD_{50} - \frac{5 - Yk}{b}$$

$$SEk = \sqrt{(SE_{50})^2 + \frac{2}{nH\,[N-H]} \times \left[\frac{5 - Yk}{b^2 i}\right]^2}$$

$$dk = LDk \pm 4.5 \times LDk \times SEk$$

For example, if the LD_{95} is desired, k would equal 95 and would be calculated accordingly:

$$\log LD_{95} = \log LD_{50} - \frac{5 - Y95}{b}$$

where Y_{95} is the probit corresponding to 95% and we can obtain it from the statistical table in which any percentage has its probit Y.

RESULTS

The results of pretest range-finding determinations are found in Table 1, and final tests LD_{50} determinations for albino and wild house mice are provided in Tables 2 and 3.

Table 1. Results of pretest range-finding determinations for three levels of zinc phospbide for albino mice and wild mice.

	Mortalities (dead/tested)	
Dose (mg/kg)	Albino mice	Wild mice
10	0/10	0/10
40	4/10	5/10
160	10/10	10/10

Table 2. The results of a series of six dose levels for LD_{50} determinations of zinc phosphide toxicity for albino mice.

	Mortalities (dead/tested)		
Dose (mg/kg)	Males	Females	Combined sexes
26.70	0/5	0/5	0/10
35.62	0/5	3/5	3/10
47.53	0/5	3/5	3/10
63.41	2/5	5/5	7/10
84.60	3/5	5/5	8/10
112.87	5/5	5/5	10/10
Total	10/30	21/30	31/60
LD_{50} (mg/kg)	73.23	38.86	53.34
SE_{50} (mg/kg)	0.04337	0.04337	0.01099
d_{50} (mg/kg)	±14.29	±7.58	±2.64

Table 3. The results of a series of five dose levels for an LD_{50} determination of zinc phosphide toxicity for wild mice.

Dose mg/kg	Mortalities (dead/tested) Males	Females	Combined sexes
20.01	0/5	0/5	0/10
26.70	1/5	0/5	1/10
35.62	3/5	5/5	8/10
47.53	4/5	5/5	9/10
63.41	5/5	5/5	10/10
Total	13/25	15/25	28/50
LD_{50} (mg/kg)	-	-	32.68
SE_{50} (mg/kg)	-	-	0.02434
d_{50} (mg/kg)	-	-	±3.58

From these results and the formulas introduced above, the regression equation for combined sexes of Swiss-Webster albino mice can be derived as follows:

$$Y = 6.78x - 6.71, \; sb = 1.0703$$

For Swiss-Webster albino mice (sexes combined), the LD_{95}, SE_{95} and its 95% confidence limit are:

$$LD_{95} = 93.42 \text{ mg/kg}$$
$$SE_{95} = 0.04413 \text{ mg/kg}$$
$$d_{95} = 93.42 \pm 18.55 \text{ mg/kg}$$

For combined sexes of wild mice, the regression equation

$$Y = 10.38x - 10.72, \; sb = 1.3139$$

and the following are obtained:

$$LD_{95} = 46.39 \text{ mg/kg}$$
$$SE_{95} = 0.03302 \text{ mg/kg}$$
$$d_{95} = 46.39 \pm 6.89 \text{ mg/kg}$$

In these toxicity determinations there were 88 mice out of a total of 170 that died of zinc phosphide poisoning and the vast majority of deaths, 85 out of 88 (97%), occurred within 48 hours after administering the zinc phosphide. Fifty-one (58%) died in the first 24 hours. Three of the wild mice died on the third day after administering the chemical. Specifics on time to death are provided in Table 4.

Table 4. Time to death of dosed animals in both the pretest range-finding and the final LD_{50} determinations.

Death time (day after gavage)	Albino mice range-finding	Albino mice LD_{50} det.	Wild mice range-finding	Wild mice LD_{50} det.
1st	12	19	10	10
2nd	2	12	5	15
3rd	0	0	0	3
Subtotal	14	31	15	28
Combined total	45		43	

DISCUSSION

In above regression equations the regression coefficients "b" represent the individual differences of the reaction of the tested animal on the rodenticide. The larger the b, the less the individual differences. From the equations it is obvious that the individual difference between albino mice (b=6.78) is larger than that between wild mice (b=10.38), but the "t" test showed that the difference is not significant (P>0.05).

The LD_{50} of both sexes was calculated separately for albino mice, and resulted in 73.23 mg/kg for males and 38.86 mg/kg for females. The statistical test shows the difference between them is highly significant (P<0.01). On the other hand, it is impossible to calculate the LD_{50} of female wild mice because there are too few affected groups (i.e., only two). This is insufficient as four groups are the minimum needed with this method of analysis. Still, we observe that the sex difference for wild mice is not significant since only 3 of the paired tested groups (the part outlined by a rectangle in Table 3) showed different mortalities for the males and females. None of these pairs show a significant difference statistically by consulting the "c" value table for fourfold table.

Bell (1972) reported an LD_{50} of 25.77 (± 12.16) mg/kg for female albino mice; however, the strain of mice used was not indicated. This differs from the 38.86 (± 7.58) mg/kg found in our determinations. Bell's smaller sample size, methods of calculation, and procedures for administering the toxicant differed from ours, thus could possibly account for the apparent difference in results.

The time to death seems to depend on two factors: the dose and the apparent individual susceptibility to the compound. The larger the dose or the apparently less resistance of the individual, the quicker the animal dies.

There are a number of methods of LD_{50} determinations. The simpler ones are not very precise and often do not provide adequate information. On the other hand, the more precise

tests require more animals and are often complicated and difficult to use. The procedure used in the this study provides the necessary data with satisfactory accuracy and does not require complex calculations.

Based on these studies, wild house mice are significantly more susceptible to zinc phosphide than Swiss-Webster albino mice. The LD_{50} for wild house mice (sexes combined) is 32.68 ($\pm$ 3.58) mg/kg and 53.34 ($\pm$ 2.64) for albino mice ($P<0.01$). Thus, if albino mice are used in place of wild house mice for the evaluation of zinc phosphide bait formulations, the results may be misleading.

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