The toxicity of some commercial lead arsenates with a comparison of methods for determination
by Robert D Eichmann

A Thesis Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Entomology
Montana State University

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Approved:

A L Strand
In Charge of Major Work

A L Strand.
Chairman Examining Committee

F. B. Cotner
Chairman Graduate Committee

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INTRODUCTION

The acid form of lead arsenate ($Pb\ H\ AsO_4$) is probably the best known insecticide, yet a comparatively small fund of knowledge is available concerning its chemical and physical properties. These in turn may greatly modify our present control practices as well as the toxicity of the poison to insects..

At the present time lead arsenate is still the best material for controlling the codling moth. It is the only arsenical which can be applied to apples and pears a number of times during the season without plant injury. Yet the residue problem has stimulated great activity in finding substitutes for it. However, in regions such as western Montana the residue problem is of minor importance because only one cover spray is required for satisfactory control. It would seem desirable to know more about the chemical, physical and toxicological properties of lead arsenate before casting it aside in favor of a more expensive method. The experiments reported here are a part of a general program at the Montana Agricultural Experiment Station dealing with acid lead arsenate.

A recent bit of research has established that the solubility of the arsenates is governed, at least in part, by the hydrogen ion concentration. If the solubility is affected by the hydrogen ion concentration likewise the toxicity to insects might be modified in the same direction, as toxicity is believed to be proportional to the soluble arsenic.

It is the purpose of this work to determine if different commercial brands of lead arsenate with significant differences in the per cent of soluble arsenic at a given pH show comparable differences in toxicity to the silk worm. Two methods for determining toxicity of stomach poisons are available. Each is applicable to the problem at hand and apparently gives significant results. Since the two methods have never been compared by one person such a comparison is to be included in this work.

ACKNOWLEDGMENT

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REVIEW OF LITERATURE

A goodly amount of work has been done on the solubility of lead arsenate in waters, in soil solutions and spray combinations. Driggers (8) gives an excellent review of the work to 1929. No literature is available concerning the effect of hydrogen ion concentration on solubility. Driggers found the solubility of acid lead arsenate was not greatly increased in bog water with a pH of 4.3 - 4.8. This is the nearest approach to a study of the effect of hydrogen ion concentration on solubility of the arsenates. In general, these workers found increased solubility on the alkaline side, least solubility in distilled water, and little change on the acid side. They were not concerned in tying up solubility with hydrogen ion concentration. No attempts were made to control salts in the water, materials in the spray tank, etc., which might also effect the solubility. Instead they took conditions as they were and gave them a superficial examination. Swingle (18) concludes that the original per cent of soluble arsenic in lead arsenate has little or no effect on insects but he does not consider how easily this initial amount can be increased.

Pepper and Green (15) were the first to do any work under controlled conditions on the effect of hydrogen ion concentration on the solubility of the arsenates. They used the official method of agricultural chemists (1) for determining the solubilities. Samples of commercial products were obtained direct from the manufacturers just prior to analysis. The pH of the samples to be tested was adjusted at eleven different concentrations with hydrochloric acid and ammonium hydroxide. One test was run in boiled distilled water. They found acid lead arsenate to be at the minimum solubility in distilled water. The pH in this case was governed by the sample and lay between pH 4 to pH 6 depending on the product. As the pH increased through neutrality to a pH of 9 the solubility increased rapidly to an average of 11.66% soluble. As the sample became more acid to pH 2.5 the solubility again increased but not so rapidly as it only averaged 2.64%. Basic lead arsenate gave about .14% soluble from pH 6 to 9 but increased slowly in solubility to 1.29% soluble at pH 3. Calcium arsenate was least soluble, .5%, at pH 11.5 and increased steadily to 41.5% at pH 4.

Pepper and Green take issue with the official method of determining solubility of acid lead arsenate in distilled water for the solubility is at its lowest. Further surface waters used in spraying are well over on the alkaline side where the solubility is much higher and in no way proportional to the amount soluble in distilled water. Likewise calcium arsenate is tested for solubility at pH of 9 whereas its minimum solubility occurs at pH 11.5, so the two procedures greatly favor acid lead arsenate in a comparison of solubilities.

In their discussion, Pepper and Green suggest that although in general the samples of lead arsenate are quite consistent their peculiarities of solubility between pH 6 and pH 8 may be rather important when related to their toxicities to various insects. Likewise there should be little difference between the absorption of calcium and acid lead arsenate in the insect gut, which in general is slightly alkaline. They state, however, that this phase of the problem awaits further development.

A search through the literature for a method to use in determining the toxicities of stomach poisons is somewhat disheartening. Each worker developed a system and then seemed to take care in expressing the results in such a form that they could not be readily compared with those of another method.

From the first use of lead arsenate attempts have been made to determine its killing power. Marlett (13) compared arsenicals on the basis of the time required to kill the insects and the total number killed. Holloway (9) took the time for Paris green to cause death, called this unity and rated other insecticides on this basis to determine the poison exponent. The time to produce death coupled with the amount of sprayed leaf surface eaten was used by Scott and Siegler (16) to compare arsenicals. They made no attempt to arrange preparations in order of toxicity as they used various quantities of poisons. Lovett and Robinson (11) considered the time required to kill, the approximate amount of arsenical necessary to produce death, and the ratio of As_2O_5 in tissues and excrement as an index to toxicity. Moore (14) found the latter method to his liking and used it in his toxicity experiments. He carried it a little further and took the reciprocal of the number found by dividing the amount of arsenic in the excreta by the amount in the tissue as the index of toxicity. Lead arsenate was almost one so he gave it this value and rated the other materials on the same basis. In this manner it is possible to give any stomach poison a rating on the lead arsenate basis. Cook and McIndoo (7) also carried on tests but these, like the earlier attempts, were really a test of effectiveness rather than toxicity.

F. L. Campbell (2,3,4), who has done more along the line of toxicity of stomach poisons to insects than any other, began publishing along this line in 1926. First he fed drops of soluble arsenicals to caterpillars which gave a good measure of toxicity but was not applicable to arsenical suspensions. Janisch (10) at this time published a method of dusting poison on leaves, weighing, feeding to insects until dead, and then reweighing to determine the amount ingested and to estimate the relative toxicity of stomach poisons. This method was very inaccurate in many ways but gave Campbell an idea which he developed to a very accurate method of determining relative toxicity of stomach poisons.

Campbell (5) dusted a thin coat of poison on leaf discs of known area and determined the amount present by dusting weighed cover classes of the same area and re-weighing them. Then he pasted another leaf disc over each dusted leaf forming a sandwich, fed these to silkworms for a short time and removed them. Periods of feeding were gauged so that the median lethal dose, at which 50% of the larvae died and 50% recovered, could be determined. Determination of dose was made by photographing the partially eaten discs, finding the area eaten with a planimeter and calculating how much poison had been ingested.

No further schemes for determining toxicities have been developed which compare in accuracy with the method of Moore or Campbell. In 1930 Campbell (6) compared his method with one by Marcovitch (12) wherein he used the time to produce 50% mortality of mosquito larvae as an index of toxicity, and the simple cage method of allowing insects to feed on poison until dead, using the time as the index of toxicity. He justly concludes that this method of determining the median lethal dose is superior. However, no one has compared this method with that of Lovett and Robinson,further developed by Moore,which seems of equal merit.

In this work either of the methods might be used to determine the toxicity of the arsenates in question. Rather than decide arbitrarily which to use, a comparison of the two methods is to be made along with the determination of the toxicity of the arsenates at different hydrogen ion concentrations and solubilities.

PROCEDURE

First it was necessary to build a dusting apparatus for the Campbell method. A stand for the bell jar was set up. A large glass tube 10 cm. long and 2 cm. wide, narrowed at both ends, and containing a small ball of paraffin, was used to hold the dust like the shortened 50 c.c. pipette Campbell used. An air compressor was available to furnish the air to carry the dust.

It was found that on lifting the jar containing the small particles of dust suspended in the air, that currents of air swirled through the jar.

This made an uneven distribution of the particles. As this was certain to make a variation in the amount deposited at different places an attempt was made to eliminate this source of error. A movable platform was suspended by springs from the ceiling. The jar, being quite heavy, depressed the springs slightly and held the board platform tightly in place before and after moving.

Trouble was experienced in perforating a cork to get an even distribution of dust. To offset this, short pieces of capillary tubing were inserted in the cork. This gave an excellent spread of dust.

About one to two grams of the powdered arsenical were placed in the glass tube which was connected to the air compressor and inserted in the cork which was placed in a hole in one side of the platform. Dust filled the bell jar as the motor of the air compressor was started. As soon as the dust was well distributed the motor was turned off. The heavy particles began settling out rapidly. This was allowed to continue for some time, then the dust tube was disconnected and the other half of the platform, bearing the leaf discs and cover glasses, was moved under the jar. This was accomplished by depressing the springs about one-half inch and sliding the platform quickly. The leaf discs and cover glasses, 7/8 inch in diameter, were allowed to remain under the dust jar until a thin coat of dust was deposited, then the platform was moved back. Coatings from .12 to .64 milligram per disc were used. Tests with single weighed cover glasses showed the deposit to be fairly uniform throughout the dusting area. A chainomatic balance accurate to .1 mg. was available for the weighing.

Determination of the amount of dust deposited was made by first weighing five undusted cover glasses and re-weighing after dusting; the increase in weight divided by the number of cover glasses gave the average amount per disc.

Sandwiches were then made by coating undusted leaf discs with fresh starch paste and pressing one of these on each dusted leaf disc. The sandwiches were placed in slit corks and one was given to each silkworm used in the test of the particular arsenical. The silkworms were allowed to feed for varying lengths of time, depending on the amount of deposit in the sandwich, the weight of the worms and the estimated toxicity of the poison being tested. In this way the remaining portion of the sandwiches differed greatly in contour when they were removed. In each test one or more worms failed to start feeding before the sandwiches wilted. These were saved as checks and in all cases lived normally. Following the test each worm was given a fresh portion of unpoisoned mulberry leaf and placed in a ventilated pill box at a constant temperature of about 27°C. to await recovery or death.

The worms for the tests were reared on mulberry leaves in a constant temperature cabinet at 27°C. Mulberry trees growing in the greenhouse allowed this work to proceed in the winter time. Fourth instar silkworms averaging about .6 g. were used in the tests.

Immediately after feeding a series of worms the sandwich remnants were arranged in order on a glass plate. This was used as a photographic negative from which prints were developed. In each case an uneaten sandwich was photographed to permit estimation of the amount of shrinkage during feeding. A polar planimeter was used on the photographic prints to calculate the portion of sandwich eaten. From the weight of the dust deposited and the area eaten the dosage was then calculated. This was further developed to milligrams of poison per gram of silkworm from the weight of each worm, determined before feeding.

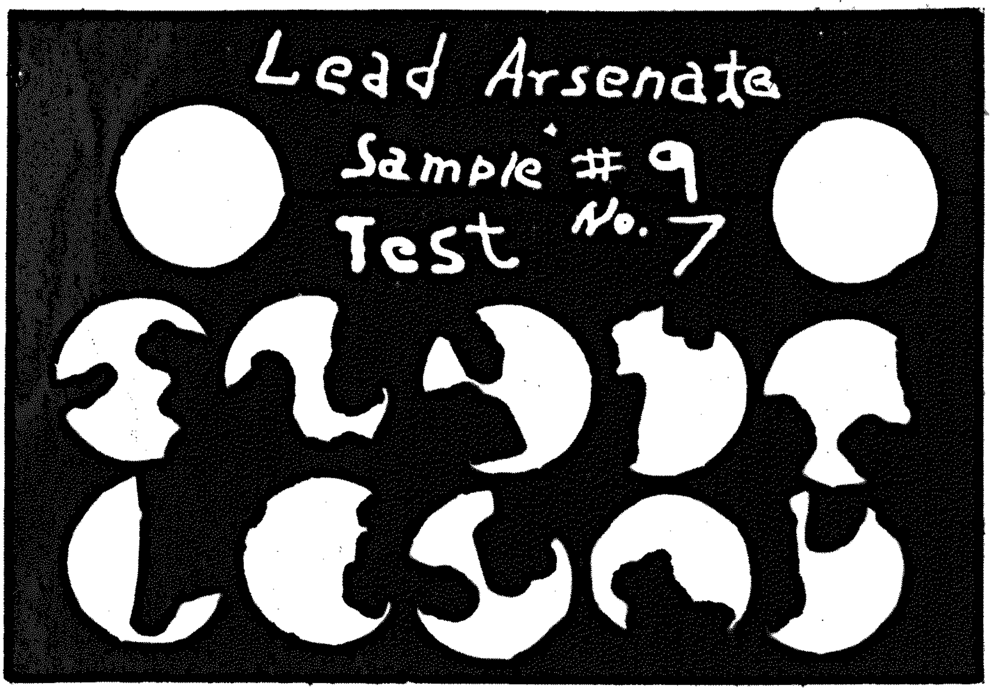

Fig.1. Sample of prints used in calculating dosages by the Campbell method.

The silkworms were allowed about 48 hours in which to die or recover. If they were not dead in that time they were sure to recover. On the basis of lethal and sublethal doses the median lethal dose was then determined. A sufficient number of worms was used for each poison so that the median lethal dose (M.L.D) could be accurately determined. The M.L.D. was then used as a basis for comparing the toxicity of the poisons in question.

The remaining portions of sandwiches for the tests on the first and second poisons were saved after photographing. They were then digested in nitric and sulphuric acid and the amount of arsenic determined by the

Gutzeit method (17) (1). The amount of lead arsenate eaten was then calculated on the basis of sandwich areas and the per cent of arsenic per unit of each lead arsenate; previously found by the iodine titration method of official agricultural chemists (1). From the amount of lead arsenate ingested the mg. per g. of insect were determined and likewise the M.L.D. for comparison with the Campbell sandwich method.

Dead worms and their excrement were collected from the pill boxes after 48 hours to be tested by the Moore method. Each worm and its excrement was digested and tested separately as with the sandwich remnants. The ratio of the arsenic in the tissues to the arsenic in the excrement was determined from the average of all lethal doses for a given poison. Moore determined his values by allowing 50 locusts to feed until death and running tests on them in groups of ten. The total number available for the above tests is far less than these but individual tests give a sufficient basis for comparison.

In like manner the excrement of worms which recovered from a dosage of lead arsenate was collected and analyzed for arsenic. It was estimated that approximately 100% of the arsenic had been eliminated in three days. From the total arsenic found in this manner and in the tests for the Moore method the M.L.D. in mg. per g. of silkworm was calculated a third time for the first two lead arsenates.

EXPERIMENTAL RESULTS

The alimentary tract of the silkworm was found by Cook and McIndoo (7) to have a pH of 7.1. Accordingly, three samples of lead arsenates showing very low, very high and moderate solubility at this hydrogen ion concentration were chosen for the following tests.

Table I. Per cent of soluble arsenic at different hydrogen ion concentrations for a series of commercial lead arsenates. (After Pepper and Green).

	pH								Tap Water	
	2.5	3	4	5	6	7	8	9	pH	% Soluble
Sample No.	% Soluble									
1	2.30	1.73	1.16	0.93	1.00	1.22	2.85	10.15		
2	6.00	1.88	1.00	0.30	0.50	3.10	6.40	10.20		
3	3.53	2.60	0.94	0.11	0.65	2.10	4.80	10.98		
4	1.34	0.40	0.10	0.09	0.15	2.22	6.20	9.63		
5	2.08	0.93	0.21	0.22	0.75	2.93	6.12	9.50		
6	1.38	0.76	0.34	0.28	0.30	2.02	6.16	12.16		
7	1.87	0.67	0.22	0.21	0.74	2.95	8.90	14.72		
8	1.82	0.92	0.51	0.35	0.44	1.50	4.96	11.14	7.2	2.36
9	2.25	1.28	0.65	0.37	0.70	3.63	9.14	13.61	6.8	2.74
10	1.22	0.71	0.27	0.19	0.40	3.30	7.62	12.52	6.9	2.35
11	3.96	2.04	0.71	0.61	1.51	3.13	7.90	11.87	7.0	3.52
12	4.82	2.41	1.10	0.78	1.06	2.20	5.22	10.20	7.1	2.18
13	2.03	0.97	0.32	0.24	0.22	0.80	6.05	13.00	7.1	1.62
14	2.80	1.40	0.65	0.39	0.20	1.26	9.40	14.56	7.2	2.41
15	2.30	1.00	0.50	0.25	0.45	3.30	7.90	10.68	7.1	1.90
Ave. % Soluble	2.64	1.31	0.57	0.35	0.60	2.37	6.64	11.66		

The table of the per cent solubility over a range of pH values for 15 samples of commercial lead arsenate is borrowed from Pepper and Green and included here to illustrate the influence of pH on solubility. Samples 4, 9 and 13 at pH of 7 yield respectively, moderate solubility 2.22%, highest solubility -3.63%, and lowest solubility -0.80%. These three materials were then fed to the silkworms as previously described.

As the silkworms hatched over a considerable period of time they did not mature at the same time. For this reason they were subjected to the tests in small groups as they matured. The data were then accumulated until

Table II. Data for determination of M.L.D. of lead arsenate sample No.4 by the Campbell sandwich method.

Test No.	Estimate of total area of leaf after feeding	Larva No.	Estimate prior to test of area to be eaten	Weight worm	Actual area leaf eaten sq.in.	Lead arsenate eaten mg.	Mg./g worm	Lethal or sublethal
9 .64 mg. lead arsenate per leaf disc	(.57 sq.in.	1	.11 sq.in.	1.1365g.	.17	.190	.167	L
	(.57	2	.08	.7978	.09	.101	.126	SL
	(.57	3	.08	.7591	.09	.101	.133	SL
	(.57	4	.10	.9323	.12	.134	.143	SL
	(.57	5	.09	.8657	.09	.101	.116	SL
	(.57	6	.06	.5698	.10	.112	.196	L
	(.57	7	.05	.4491	.04	.045	.100	SL
	(.57	8	.04	.4163	.06	.067	.160	SL
	(.57	9	.05	.4398	Failed to eat			
	(.57	10	.05	.5030	.06	.067	.133	L
10 .30 mg. lead arsenate per leaf disc	(.60	1	.30	1.1751	.29	.145	.123	SL
	((.60	2	.24	.8183	Failed to eat			
	(.60	3	.20	.7358	.26	.130	.176	L
	(.57	4	.20	.7498	.19	.100	.133	SL
	(.57	5	.21	.7656	.20	.105	.137	SL
	(.57	6	.20	.7417	.24	.126	.169	L
	(.57	7	.18	.6810	.21	.110	.161	L
	(.57	8	.19	.7011	.25	.131	.186	SL
	(.57	9	.17	.6356	.20	.105	.165	L
	(.57	10	.17	.6705	.17	.089	.132	SL
	(.57	11	.16	.5785	.18	.094	.162	L
	(.57	12	.16	.5637	.19	.100	.177	SL
	(.57	13	.14	.4660	.16	.084	.180	L

the final results given in Table III were obtained.

The data obtained for each silkworm in the tests on sample No.4 by the sandwich method is included in Table II to show the data used in finding the median lethal dose. Twenty-four silkworms were used in determining the M.L.D. for sample No.13; twenty-seven were used for sample No.9, and twenty-one for sample No.4.

It is not necessary to use 40 insects per test and run two tests to determine the M.L.D. as Campbell did. As the worms were developing slowly and in small numbers it was necessary to feed doses lying in or very near the median range so as not to waste specimens in the sublethal or lethal ranges. Following the weighing of the worms and the dusted leaves the area to be eaten by each worm was estimated as he fed on the sandwich. Table II includes the estimated and actual areas eaten. The value of this procedure is brought out in Table III where it is shown that out of 21 worms fed with this poison 17 lie in the median range. In other words only four are lost, whereas Campbell lost about 70 to get 10 in the median range.

Table III. Doses fed to determine the M.L.D. for the three samples of lead arsenate. Underscored figures indicate M.L.D., sandwich method.

Sample No.9				Sample No.4				Sample No.13		
Lethal	Median		Sublethal	Lethal	Median		Sublethal	Lethal	Median	
	Died	Recovered			Died	Recov.			Died	Recov.
.29	.18	.18	.10	.20	.18	.19	.12	.29	$\underline{.10}$	$\underline{.10}$
.27	.18	$\underline{.16}$	.10		.18	.18	.12	.27	.10	.10
.24	$\underline{.16}$	.11	.09		.17	$\underline{.16}$	.10	.25		$\underline{.10}$
.23	.15		.09		.17	.14		.19		$\underline{.10}$
.23	.15		.08		.17	.14		.13		
.22	.14		.08		$\underline{.16}$	.13		.12	.06?	
.20			.07		$\underline{.16}$	.13		.11		
.20			.07		.13	.13		.11		
.20						.13		.11		
.19										

Table III gives the median lethal dose for the three samples by the sandwich method. These doses are included in Table IV for comparison with the per cent of soluble arsenic at pH 7 and the per cent of total arsenic.

TABLE IV. M.L.D. by sandwich method tabulated with arsenical content of the three lead arsenates.

Sample No.	Per cent soluble arsenic at pH 7	Per cent of total arsenic	M.L.D. in Mg./g of silkworms
9	3.63	28.2	.16
4	2.22	25.3	.16
13	0.80	28.4	.10

The data from Tables III and IV show little correlation. Sample No. 13 is very much more toxic but the greater toxicity is not associated with per cent of total arsenic nor with per cent of soluble arsenic except that sample No. 13 has the lowest amount of soluble arsenic.

In an effort to check the accuracy of the Campbell sandwich method with the equipment available, the M.L.D. for the first two samples of lead arsenate was determined in two other ways. As previously mentioned, Gutzeit determinations were made of the arsenic on the sandwich remnants and in the excrement and tissues of the silkworms. This was calculated in terms of milligrams of lead arsenate per gram of silkworm for comparison.

Table V gives a tabulation of these results. Little or no correlation can be found. In some cases the determination by all three means lie within .01 mg. per gram of each other. Other times the dosages may lie over a range of several hundredths. The first five doses in sample No. 13 which give the greatest variation need no consideration for they are well

Table V. Dosages determined by three methods.

			Sample No. 9		
Test No.	Worm No.	Sandwich method	Gutzeit test on sandwich remnant	Gutzeit test on excrement or excrement plus tissue	Lethal or sublethal
6	3	.111	.134	.118	SL
	4	.077	.087	.073	SL
	5	.074	.084	.123	SL
	6	.097	.089	.101	SL
	9	.090	.086	.079	SL
	10	.079	.069	.086	SL
	11	.100	.070	.158	SL
7	1	.186	.155	.161	L
	2	.232	.219	.087	L
	3	.182	.285	.237	L
	4	.183	.262	.204	L
	5	.243	.125	.211	L
	6	.228	.144	.206	L
	7	.165	.076	.231	L
	8	.224	.181	.175	L
	9	.138	.070	.154	L
	10	.292	.286	.150	L
8	1	.073	.030	.060	SL
	2	.154	.083	.185	L
	3	.150	.069	.133	L
	4	.162	.066	.071	SL
	5	.199	.079	.217	L
	6	.177	.133	.075	SL
	7	.094	.042	.053	SL
	8	.205	.079	.259	L
	9	.200	.096	.117	L
	10	.275	.161	.210	L

Table V, continued.

Sample No. 13					
Test No.	Worm No.	Sandwich method	Gutzeit test on sandwich remnant	Gutzeit test on excrement or excrement plus tissue	Lethal or Sublethal
1	1	.294	-	.828	L
	2	.268	-	.473	L
	3	.247	-	.584	L
2	1	.063?	-	.157	L
	2	.112	-	.197	L
	3	.113	-	.127	L
3	1	.042	.035	.044	SL
	2	.044	.049	.181	SL
	3	.027	.028	.149	SL
	4	.103	.120	.302	L
4	1	.065	.114	.034	SL
	2	.099	.152	-	SL
	3	.084	.098	.091	SL
	4	.026	.013	-	SL
	6	.053	.073	.061	SL
	7	.098	.086	.093	SL
5	2	.106	.111	.104	L
	3	.189	.210	.163	L
	4	.097	.078	.017	SL
	5	.128	.120	.150	L
	6	.111	.089	.097	L
	7	.087	.063	.036	SL
	8	.104	.107	.056	SL
	9	.119	.162	.131	L

over in the lethal range. The results show that the check with Gutzeit determinations is no more accurate, if as accurate, as the sandwich method,; otherwise the two Gutzeit calculations would check closer.

The discrepancies brought out in the comparison in Table V are further accentuated in Table VI where the M.L.D. is determined for each method in both samples. It seems quite apparent in Sample No. 13 that the M.L.D. of .10 by the sandwich method is low. An average of the M.L.D. for the three methods gives .113 mg. per g.

In sample No. 9 the M.L.D. by the Campbell method appears high. An average M.L.D. for the three methods gives sample No. 9 a M.L.D. of .123 mg. per g.

No doubt all three methods as used in this experiment are subject to considerable error but a M.L.D. of .11 mg. per g. fpr sample No. 13 and .12 mg. per g. for sample No. 9 appear reasonable. At least sample No. 13 is more toxic than sample No. 9. One might consider dropping the sandwich method entirely in the determination but it has already been shown that the Gutzeit tests may vary in either direction when compared with each other. They are too variable for more than checks on the approximate value. This statement becomes more clear in view of the following.

If a worm weighing about .5 g. were digested and tested for arsenic by the Cutzeit method and found to contain .001 mg. of As_2O_3 the figure will not even be significant as it lies in the error of the method. However, in stating that value in terms of mg. of lead arsenate per gram of silkworm it becomes .006. Therefore the method used here is accurate only to about .01 mg. Further error lies in fixing the end point of an unknown stain to within .001 mg. when standard stains themselves are not constant.

Table VI. Comparison of M.L.D. determined by three means

Sample No. 13

Sandwich method				Dosage calculated from Gutzeit test of sandwich remnants				Dosage calculated from Gutzeit test of tissue and excrement			
Lethal	Median		SSL	Lethal	Median		SL	Lethal	Median		SL
	Died	Recov.			Died	Recov.			Died	Recov.	
.29	$\underline{.10}$	$\underline{.10}$	.09	.21	.16	.15	.08	.83	.20	.18	.06
.27	$\underline{.10}$	$\underline{.10}$	.08		.12	$\underline{.11}$	.07	.58	.16	.15	.06
.25		$\underline{.10}$	.06		.12	$\underline{.11}$	.06	.47	.16	.09	.04
.19		$\underline{.10}$	.05		$\underline{.11}$	.10	.05	.30	.15	.09	.04
.13	.06?		.04		.09	.09	.04		$\underline{.13}$		.03
.12			.04				.03		$\underline{.13}$		.02
.11			.03				.01		.10		
.11			.03						.10		
.11											

Underscored figures indicate the apparent M.L.D. by each method of determination. Figures are in milligrams of lead arsenate per gram of silkworm.

Sample No. 9.

.29	.18	.18	.10	.29	.12	.30?	.04	.26	.16	.16	.08
.27	.18	$\underline{.16}$	.10	.29	.10	.13		.24	.15	$\underline{.12}$	.08
.24	$\underline{/16}$	.11	.09	.26	.08	.13		.23	.15	$\underline{.12}$	.08
.23	.15		.09	.22	.08	$\underline{.09}$		.22	.13	.10	.07
.23	.15		.08	.18	.08	$\underline{.09}$		.21	$\underline{.12}$	.09	.06
.22	.14		.08	.16	.08	$\underline{.09}$		.21	.09		.05
.20			.07	.15	.07	.08		.21			
.20			.07	.14	.07	.07		.20			
.20						.07		.18			
.19						.07		.17			

The Lovett-Robinson-Moore method of determining toxicity of an arsenical by the ratio of the arsenic in the tissue to the arsenic in the excrement also makes use of the Gutzeit method for arsenic determination. The ratio was determined for each lethal dose in samples 9 and 13 from the Gutzeit tests run in checking the Campbell method. Such a procedure certainly assails the significance of the method, for the ratio ranges from .200 to 2.000 in individual cases. By taking the average of 50 insects in five tests such variation is not apparent, but what is the value of the method? There is no constant relationship.

Table VII. Ratio of arsenic in tissue to arsenic in excrement

Sample No.	% Total arsenic	% Soluble arsenic	Average mg. As_2O_3 in tissue	Average mg. As_2O_3 in excrement	Ratio	No. worms
9	28.2	3.63	.018	.011	.6111	16
4	25.3	2.22	.008	.020	2.5000	8
13	28.4	0.80	.013	.014	1.0761	12

The ratio for sample No. 4 was determined from the average of eight worms run in two tests.

DISCUSSION AND CONCLUSIONS

The Campbell sandwich method allows error to develop in the dusting of the leaf discs. In moving the leaf discs under the dust jar currents of air are bound to disturb the even distribution of dust on the discs. The balance available for this work was accurate only to .1 milligram but by weighing five cover glasses instead of two this source of error was greatly reduced. In order to use this method satisfactorily one should have a balance accurate to .001 milligram, to determine if the distribution of dust is even on discs placed under various parts of the jar and to increase the accuracy of the actual weighing. With such accuracy slight differences in toxicity could be readily determined.

The ability to make sandwiches and to measure photographic prints of leaf remnants with a planimeter is readily acquired with practice, and contributes very little to the error of the method.

The Campbell method is very tedious, entailing a great deal of manipulation and calculation to determine the M.L.D. for a single substance. The very fact that it is so intensive increases its value, for a very small number of insects (about 20) are sufficient for determining the M.L.D., if care is used in feeding. The Campbell method is also applicable to all stomach poisons and all chewing insects which eat the edge from leaves. It affords an excellent laboratory method for determining toxicity.

The ratio of arsenic in the tissue to arsenic in the excrement is of little value as an index of toxicity. Individual tests have shown a variation of .200 to 2.000 in the ratio. Of course this does not appear in the average for a large series of insects, but what is the value of an average based on such wide variables? Further, the method applies only to arsenical compounds and depends on the Gutzeit test which in itself is quite variable, especially in the small amounts to be tested in insect toxicology. Moore's excellent table of values for arsenicals might be quite different if he had repeated his experiments.

The accuracy of the results of these experiments are open to question due principally to the scales used. However, there is undoubtedly a considerable difference in toxicity of sample 13 as compared with samples 9 and 4, at least to the silkworm. The numerical values obtained directly from the sandwich method and the average with the Gutzeit tests are of importance only as showing a difference. Samples 4 and 9 are no doubt very similar in toxicity.

It is impossible to correlate toxicity with per cent of soluble arsenic or per cent of total arsenic in this experiment. The indication, however, is that the toxicity of lead arsenate decreases with an increase in the per cent of soluble arsenic. The per cent of total arsenic appears to have no bearing on the toxicity of these samples.

Campbell found the M.L.D. for acid lead arsenate to lie between .08 and .10 in a series of tests and calls .09 mg./g of silkworm the M.L.D. He used a sample of known purity whereas commercial compounds vary in their composition. With a balance accurate to .001 milligram, undoubtedly commercial samples of lead arsenate will be found to vary in toxicity, as indicated by this experiment. Possibly the relation of hydrogen ion concentration to the per cent of soluble arsenic has no bearing on toxicity due to other chemical effects encountered in the insect stomach.

SUMMARY

Three brands of commercial lead arsenate exhibiting wide differences in the per cent of soluble arsenic at a pH value of 7, are tested for toxicity to the silkworm as its alimentary tract shows that hydrogen ion concentration.

A review of the literature shows only one paper dealing with hydrogen ion concentration as it effects the per cent of soluble arsenic.

Methods for determining toxicity of stomach poisons are also reviewed.

The Campbell "sandwich" method and the Lovett-Robinson-Moore method employing the ratio of arsenic in the tissue to arsenic in the excrement are used for determining toxicities.

The arsenical ratio method is found to be based on such variables that its value as an index of toxicity is questionable.

The sandwich method is checked with Gutzeit arsenic determinations and found to be more accurate than the Gutzeit test. If a microbalance were used it could determine very slight differences in toxicity of stomach poisons.

No correlation is found between the per cent of total arsenic and toxicity. The sample showing the least per cent of soluble arsenic is the most toxic, indicating that the problem is complicated with other factors. However, the fact remains that commercial lead arsenates do differ in toxicity.

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