UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

Research Bulletin 183

Some Results from Feeding Spray Chemicals to Albino Rats

T. J. TALBERT and W. L. TAYLOE

(Publication Authorized March 8, 1933)

COLUMBIA, MISSOURI

MARCH, 1933

UNIVERSITY OF MISSOURI | COLLEGE OF AGRICULTURE

Agricultural Experiment Station

CONTENTS

	Page
Abstract	4
Acknowledgment	4
Introduction	5
Determining Amount of Arsenic on Apples	5
Factors Influencing Amount of Residue	5
Spray Applied with Spreaders and Stickers	5
Results from Analyses	6
Arsenical Residue in Calyx and Stem Ends of Apples	6
Analyses of Apples Wrapped and Packed	7
Preliminary Investigations; 1930	7
Materials and Methods	7
Lethal Dose	9
Comparative Doses for Man and for Rat	10
Investigations; 1931-1932	10
Determining Dosages	12
Discussion of Results	14

(Original Rat Stock)

Rats Receiving Lead Arsenate	14
Rats Receiving Calcium Arsenate	14
Rats Receiving Soluble Lead Arsenate	14
Rats Receiving Arsenic Trioxide	14

(First Generation of Offspring)

Rats Receiving Lead Arsenate	15
Rats Receiving Calcium Arsenate and Lead Acetate	15
Rats Receiving Soluble Arsenic Trioxide	15

(Results from Larger Dosages)

Rats Fed 200 Times World Tolerance	15
Post Mortem Examination	18
Mortality	18
Literature Cited	19

ABSTRACT

Arsenic as arsenic trioxide or in combination with the lead in the form of commercial lead arsenate, or with calcium arsenate and lead acetate, appears to affect albino rats as follows:

Arsenic salts in quantities ranging from the equivalent of four times to two hundred times the official world tolerance may promote activity and growth in the original stock for the first twenty-three to twenty-five weeks.

Dosages of insecticides continued for periods of more than fifteen weeks decrease the ability to raise young in the first generation.

Arsenic did not appear to retard growth unless fed in quantities larger than the equivalent of .04 grain arsenic trioxide per pound of fruit.

Lead as lead acetate and arsenic in its various forms as an insecticide appear to have injurious effects on albino rats only after prolonged feeding.

Arsenic or lead, or both, when fed in quantities larger than the equivalent to .04 grain for about 175 days, seemed to have an injurious effect on the offspring by decreasing the weight and the ability of the females to produce and rear young.

When spray chemicals are fed daily over long periods in quantities equivalent to more than four times the world tolerance, they have very marked injurious effects on albino rats, and when the feeding is extended for four hundred days or more, the mortality rate increased and amounted to as much as 35.4 per cent.

Contrary to general opinion, the spray insecticides do not have as acute toxic effects on albino rats as is generally supposed, even when used in amounts of two hundred times the official world tolerance.

Our experience indicates that the arsenicals in fruit sprays have, in fact, acute stimulating effects, and injurious effects are brought on only when feeding is regular and prolonged, as shown in the feeding periods ranging from 378 to 497 days.

If it may be assumed that the spray chemicals have an effect upon man similar to that which they have upon albino rats, it is the opinion of the authors that there is little likelihood of a human consuming as spray residue on apples, sprayed and handled in the usual manner, enough arsenic either at one time or over an extended period to be injurious. In view of the varying effects of lead poisoning given in the literature, the tests made in this experiment do not appear adequate to justify the drawing of conclusions as to the risk of lead poisoning from the consumption of sprayed apples.

Some Results from Feeding Spray Chemicals to Albino Rats

T. J. TALBERT and W. L. TAYLOE

In the control of injurious insects such as the codling moth of the apple, the application of sprays may in some sections leave upon the fruit an arsenical residue in excess of the world tolerance of one one-hundredth grain of arsenic trioxide per pound of fruit. This may be true particularly in the irrigated districts of the country and where as many as nine or more spring and summer arsenical sprays are applied. Furthermore, the spray residue is made more difficult to remove when oil or other adhesive materials are added for greater insecticidal efficiency.

It is now common knowledge that the United States Food and Drug Administration of the Department of Agriculture does not permit foreign or inter-state shipments of apples which contain an arsenical residue of more than the world tolerance. The world tolerance was also made the domestic tolerance in 1932. This so-called tolerance or amount of arsenic trioxide permitted upon the fruit, 0.01 grain per pound of fruit, was apparently arrived at more or less arbitrarily; that is, without adequate scientific information or evidence. In other words, the tolerance, from the information we now have, might have been placed just as properly somewhat lower or somewhat higher. The writers, therefore, have undertaken to secure experimental evidence to show the toxicity of spraying chemicals.

DETERMINING AMOUNT OF ARSENIC ON APPLES

Factors Influencing the Amount of Arsenical Residue.—The total amount of arsenical residue found on the fruit varies with the variety of apple, dosage of arsenicals, number of applications, spraying practices, weather conditions, and picking date. Late July or August sprays applied for the control of codling moth may increase the residue at harvest time. As the spray residue varies so much it is usually unwise to attempt to give any definite interval of time that must elapse between the last spray and the picking date in order that the residue on the fruit may be below the tolerance.

Sprays Applied with Spreaders and Stickers.—In an experiment to determine the maximum amount of lead arsenate that can be sprayed upon the fruit and retained by it after carefully harvesting, the following results were secured:

Using 2 pounds of lead arsenate with 2 pounds of calcium caseinate as a spreader and sticker to 50 gallons of water, it was found that .08 grain of arsenic per pound of fruit was retained. When 2 pounds of lead arsenate were used in bordeaux 4-6-50, .13 grain was left on the fruit.

In another spray combination where lead arsenate was again used at the rate of 2 pounds to 50 in bordeaux 4-6-50 with dormant oil at the rate of ½ gallon to 50, a residue of .05 grain remained on the fruit.

The sprays were applied about 10 days before the fruit was harvested. No rain fell upon the fruit preceding the harvesting time. Moreover, every precaution was taken in picking and handling the apples to prevent the removal of the spray residue.

Results from Analyses.—The Missouri State Food and Drug Department in making 81 analyses of 10 different varieties of apples in 1931 found that only 8 samples were above the world tolerance. This is significant because more sprays than usual were applied and there was less rainfall than the average during the growing season. The apple samples came from orchards located mainly in Central Missouri where an average of from 6 to 7 sprays were applied.

In 1932 the Missouri State Food and Drug Department made 124 analyses of apple samples. Orchard run apple fruit samples were taken and analysed a few weeks before and during the harvesting period. The work was confined mainly to Southwest Missouri where an average of from 7 to 9 spring and summer spray applications were made. Representative samples were secured and about 10 different varieties common to that section were included. Since about one-half to two-thirds of the samples analysed had been sprayed with oil as a spreader and sticker, the analyses showed a higher percentage of the samples with an arsenical residue above the tolerance than for the year 1931. The final results with the 124 samples gave 90 slightly above the tolerance and 34 below it.

Arsenical Residue in Calyx and Stem Ends of Apples.—The total residue deposit held in the stem and calyx ends of apples has been considered. Notable among those investigating the problem are Streeter, Chapman, Harman and Pearce[2]. According to these authors, several analyses were made soon after harvest to determine the distribution of the arsenical residue on apples.

Their results show that the average amount of arsenic in the ends of apples is approximately 39 per cent of the total, but it varied from 23 to 55 per cent for the samples analysed. That is; apples with a total of .03 to .04 grain of arsenic per pound may have more than the tolerance amount of .01 grain in the stem and calyx ends. It is also true that small apples may carry more per pound than large ones, because of the greater surface area and more stem and calyx ends per unit weight.

This seems important because the calyx and stem ends of the apple are rarely eaten when apples are consumed from the hand or peeled for cooking. It is important, therefore, to consider the average amount of the total residue found in the calyx and stem ends of apples as these parts are usually discarded.

ANALYSES OF APPLES WRAPPED AND PACKED

A chemical analysis to determine the amount of the arsenical residue on four samples of Jonathan and eight of Golden Delicious apples sprayed seven times, picked and wrapped, and held in cold storage until January 15, gave the following results:

Jonathan samples Nos. 1, 2 and 4 contained .004 grain and sample No. 3 contained .0045 grain of arsenic per pound of fruit, while five samples of Golden Delicious showed .004 and two .005 grain per pound of fruit.

PRELIMINARY INVESTIGATIONS; 1930

To determine the effects of insecticidal sprays upon animal life, a preliminary study using albino rats was begun in 1930. The spraying chemicals employed and fed to the rats were arsenate of lead, barium fluosilicate and cryolite.

The rats were divided into three series. In Series I the first group was fed lead arsenate, the second cryolite, and the third barium fluosilicate. The amounts fed were comparable to or greater than the quantities of arsenic trioxide which may be found on unwashed sprayed fruit. Different lots of rats designated as Series II and III were fed increasing amounts of arsenate of lead in order to determine lethal amounts. The amounts of insecticides fed were based upon the international tolerance. In all, 36 rats were employed in this preliminary investigation.

The rats used in Series I and II were 28 days old, while 3 of those used in Series III were 70 days old, and 5 were 31 days old at the time the feeding experiment began. Series I were fed insecticides for 12 weeks, Series II for 8 weeks, and Series III for 2 weeks. The insecticides were given to Series I and II at about 4:00 p. m. daily, and to Series III at 8:00 a. m. daily.

Each rat was caged separately in a nine-inch round cage. One-half-pint glass jars were employed as containers for both food and water. A balanced ration consisting of whole wheat flour, dried whole milk, and salt was kept in jars before the rats at all times. Distilled water was used exclusively as drinking water for the rats. All containers were cleaned and sterilized twice each week and the cages were cleaned three times each week.

Materials and Methods.—As a basis for establishing dosages, the international tolerance of 0.01 grain of arsenic per pound of fruit was used. Moreover, the dosage was based on relative body weight, using 60 kilograms as the weight of a human. The rats in the first series received one, two, and three times the international tolerance. As a base of reference, the per kilogram dosage was calculated correspondingly to that of a 60 kilogram man taking 0.01 grain of arsenic per day (the amount legally tolerated in one pound of fruit). Series of rats received multiplies of this per kilogram dosage, which will be referred to hereafter as so many tolerances.

The chemical analysis of the manufacturer states that 30% of the lead arsenate consists of arsenic trioxide. This figure was used in making the determinations. The same weight of barium fluosilicate and cryolite was employed in the investigations.

For the first three weeks in Series I all the insecticides were given dry in a small amount in the regular diet of the rats. After this three-week period, however, a change was made to a liquid dose given by mouth, as this appeared to be a more careful and effective method of

TABLE 1.—DOSAGES FOR THE DIFFERENT GROUPS OF RATS KNOWN AS SERIES I, II, AND III

Series No.	Age of rats	No. of rats	Dosage	Feeding Period
I	28 days	6	1 × International tolerance	12 weeks
I	28 days	6	2 × International tolerance	12 weeks
I	28 days	6	3 × International tolerance	12 weeks
II	28 days	2	2 × International tolerance	8 weeks
II	28 days	2	6 × International tolerance	8 weeks
II	28 days	2	12 × International tolerance	8 weeks
II	28 days	2	18 × International tolerance	8 weeks
II	28 days	2	24 × International tolerance	8 weeks
III	31 days	1	1.857 mg. per kilogram	2 weeks
III	31 days	1	2.785 mg. per kilogram	2 weeks
III	31 days	1	5.671 mg. per kilogram	2 weeks
III	31 days	1	8.456 mg. per kilogram	2 weeks
III	31 days	1	22.284 mg. per kilogram	2 weeks

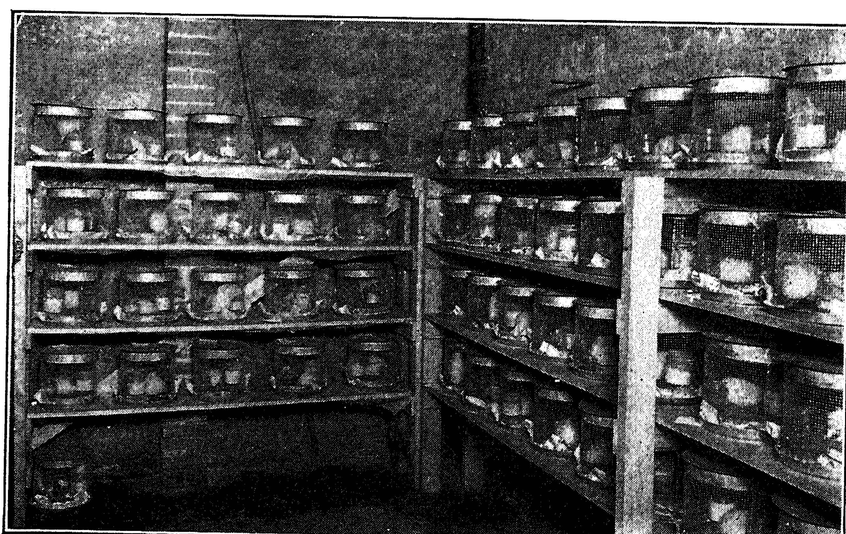

Plate I.—View of a portion of the rat feeding investigational room showing the set-up of the experiment.

feeding. It was also believed that mouth feeding eliminated the danger of individual rats failing to receive the entire amount of the insecticide. Six doses were given per week.

To make comparisons in gain of weight of the various lots, the weights of males and females were separated, the normal growth rate of males being greater than that of females. Neither the kind of insecticide nor the amount of insecticide seemed to affect the growth as measured by gain in weight. A rat receiving 24 times international tolerance (.2628 mg. per kilogram) made the greatest gain of any rat that received lead arsenate for eight weeks. Rats receiving lead arsenate made greater gains on an average than did rats receiving none.

No symptoms of poisoning were noticed at any time in any of the rats of Series I and II. In fact, normal activity and health were exhibited at all times by all the rats receiving the insecticides. An autopsy also showed no injurious effects whatever.

For Series III, however, an autopsy on each of the five rats which received from 1.857 to 22.284 mg. of arsenic trioxide per kilogram of body weight, showed a lack of internal fat compared with normal rats. The heart, kidneys, liver and intestines did not show any abnormal conditions, except in the case of one rat which received 2.775 mg. In this instance a slight degeneration of the kidneys had occurred, although it could not be certain that this was due to the arsenic.

Lethal Dose.—Three rats were used in this test. They received from 44.568 to 487.5 mg. of arsenic trioxide every 24 hours per kilogram body weight. One rat was given 44.568 mg. the first 24 hours; the second 24 hours this rat received two doses of 44.568 mg. 8 hours apart, or a total of 89.136 mg.; the third 24 hours 3 doses of 44.568 mg. given at 4-hour intervals, or a total of 133.704 mg. This last amount was continued daily until the rat died on the eighth day. This particular rat received a total of 867.02 mg. of arsenic trioxide per kilogram of weight in the 8 days before death occurred. A second rat was given 185.713 mg. per kilogram weight in a single dose at 24-hour intervals until death occurred. The rat in this test died at the end of 60 hours, and had received a total of 557.1 mg. of arsenic trioxide per kilogram body weight.

A single dose of 487.5 mg. was given another rat which died after 30 hours. This dosage is about equal to 7.5 grains of arsenic per kilogram body weight in one pound of fruit.

TABLE 2.—LETHAL DOSAGES

Series No.	Age of rats	No. of rats	Dosage	Feeding Period
III	10 weeks	1	487.5 mg. per kg.	30 hours
III	10 weeks	1 *	44.568 mg. per kg.	192 hours
III	10 weeks	1 ×	185.713 mg. per kg.	60 hours

* Received nineteen doses. × Received three doses.

Less arsenic trioxide was required to kill in a single dose than when administered in several doses over a period of time. This may in part be due to excretion, since chemical analysis of the urine and feces shows that elimination of arsenic occurs by these channels.

If there were any cumulative injurious effects of the arsenic for the period of feeding in Series I and II it was not noticeable in the behavior of the rats or in the autopsy examinations.

Comparative Doses for Man and the Rat.—From this preliminary work, we may roughly make approximations as to fatal doses of arsenate of lead found on apples as spray residue. In this, of course, it is assumed that man and the rat are affected in a similar manner.

Assuming that a pound of fruit contains two times the international tolerance of 0.01 grains (.65 mg.) arsenic trioxide per pound of fruit, a rat weighing 200 grams would be required to consume at one time .29 pounds of fruit to cause any deleterious effects. Making a similar calculation, man would be required to consume at one time 100 pounds of fruit to cause harmful effects.

The above calculations are made on the basis of the lowest quantity of arsenic trioxide required to cause deleterious effects to the rat. It should also be added that in these investigations the deleterious effects were taken at 1.857 mg. per kilogram weight.

According to O'Kane, Hadley, and Osgood[1], the minimum fatal dose for man is about 130 mg. This is much lower than would seem to be the fatal dose according to the investigations made with rats in these experiments. Moreover, these authors state that the solubility of arsenic trioxide is much less as lead arsenate than as white arsenic. It is also true that the solubility of white arsenic is variable. The solubility of the arsenic trioxide in the lead arsenate used in this investigation may be different from that used by the New Hampshire workers.

INVESTIGATIONS; 1931-1932

To obtain more exact information as to the toxicity of the spray residue found on fruit, the work reported by Talbert and Tayloe[3] was continued for a period of 497 days, March 1931 to July 1932 inclusive. In determining as nearly as possible the effect of arsenic and lead on albino rats, quantities considerably higher than those found on normally sprayed fruit were used. The amounts employed were based upon the content of arsenic trioxide.

Fifty-four albino rats 28 days old were used in beginning the work. A very accurate and easy method of feeding the chemicals to the rats was devised. This consisted of the use of a syringe and a small piece of

[1]Hartman, Henry, and R. H. Robinson. Tests show how to remove spray residue.

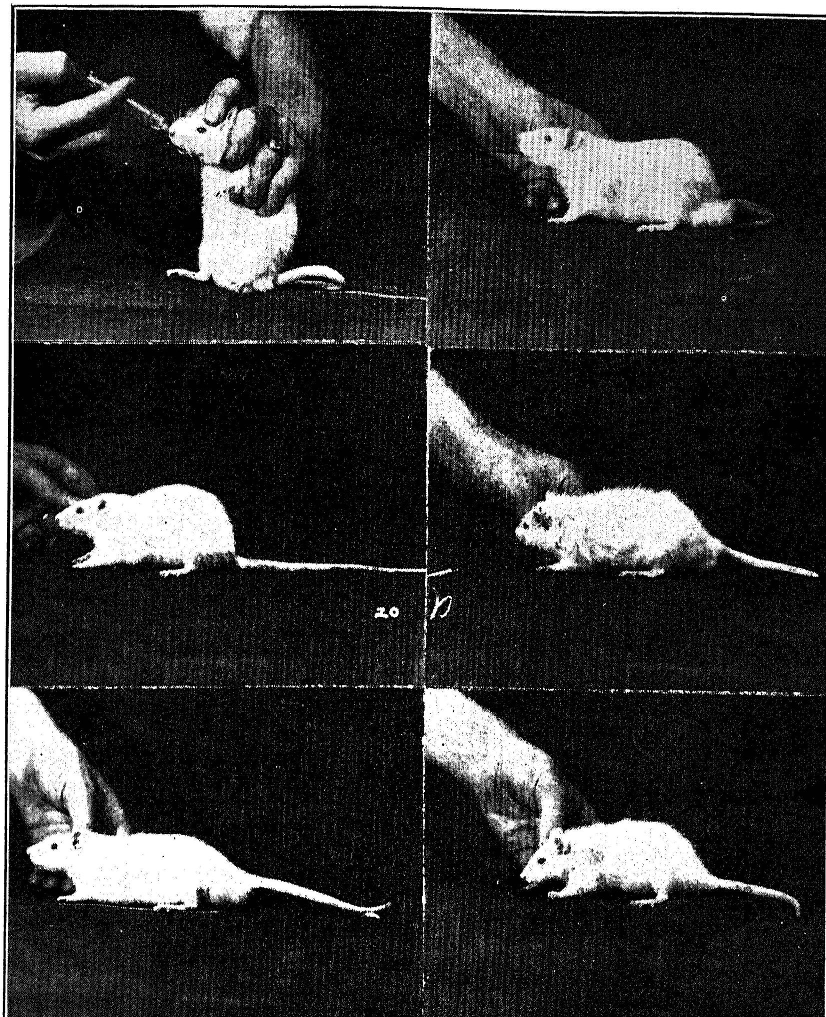

Plate II.—Some of the Animals Used in the Investigation. (Legends refer to figures directly above them, in the same order from top to bottom.)

Method used in feeding spray chemicals to rats.

Rat No. 3444L. Female
Weight 240 grams. Fed lead acetate 4 times tolerance for 71 weeks.

Rat No. 3446. Female.
Weight 265 grams. Fed lead arsenate 4 times tolerance for 71 weeks.

Rat No. 3449. Male.
Weight 390 grams. Fed lead arsenate 8 times tolerance for 71 weeks.

Rat No. 3481R. Male.
Weight 285 grams. Fed lead acetate 8 times tolerance for 71 weeks.

Rat No. 100003R. Male.
Weight 330 grams. Fed lead arsenate 200 times tolerance for 54 weeks.

rubber tubing. The liquid was drawn into the syringe and the tube inserted in the mouth and pushed down the throat of the rat about one inch. The contents of the syringe were then forced through the tubing.

Each rat was caged separately and a balanced ration was fed. Full feedings were given and at all times distilled water was supplied. The food was weighed out carefully and mixed once each week. The spray chemicals used in the investigation were lead arsenate, calcium arsenate, arsenic trioxide and soluble lead acetate. Each rat received its dosage of spray material at approximately three o'clock each afternoon, six days a week. Body weights were taken every Saturday, the amount of food consumed by each rat per week was recorded, and a fresh supply of food was given.

Each rat weighing approximately 40 grams when 28 days old was given its first dose of insecticide. The dosages were continued six days per week until the end of the feeding period. The body weight and food consumption record was taken and recorded each week. An effort was made to divide the rats into groups containing an equal number of males and females. The lots of 54 and 34 rats were separated into five divisions and insecticides were administered as shown in Tables 3 and 4. Attention is also called to Tables 7 and 8.

An effort was made to produce from the original stock four litters from each breeding female. This was attained to the extent that an average of 3.36 litters were actually produced from each female. Furthermore, approximately 50 per cent of the progeny of the original lot of rats reached an age of 28 days.

DETERMINING DOSAGES

Doses were figured on comparative weights of the white rat and the human being (human weight 60 kilograms). International tolerance (world tolerance) was considered .01 grain (or .65 milligram) of As_2O_3 as a unit for determination. Lead arsenate was considered 30% As_2O_3, which was the manufacturer's guarantee.

To secure the size of dose of lead arsenate, the percentage of lead found in lead arsenate (acid form) was determined. Atomic weights were used to determine the percentage. This determined quantity was fed in the form of lead acetate.

Calcium arsenate was based on As_2O_3, the quantity used, as that percentage in calcium arsenate.

Dosages were increased as the rats increased in weight. The basis for this increase was 25 grams body weight. Moreover, doses were increased when the weights exceeded 25 grams body weight and mutiples thereof.

For example, a rat weighing 100 grams would receive for a 4 times tolerance .00433 mg. The calculation follows:

$$\frac{100 \times 4 \times .65}{60 \times 1000} \text{ equals } \frac{.13}{30} \text{ or } .00433 \text{ mg.}$$

TABLE 3.—RAT INSECTICIDE FEEDING INVESTIGATION SHOWING DIVISIONS MADE, INSECTICIDE USED, DOSAGE TIMES WORLD TOLERANCE, NUMBER OF RATS, AND SEX

(Original Rat Stock 54 in Number—Fed 497 Days)

Division	Spray chemical		Dosage times tolerance	Males	Females
I	Lead Arsenate	×	4	3	1
	Lead Arsenate	×	5	2	2
	Lead Arsenate	×	8	1	3
II	Calcium Arsenate	×	4	2	2
	Calcium Arsenate	×	5	3	1
	Calcium Arsenate	×	8	2	2
III	Lead Acetate	×	4	2	2
	Lead Acetate	×	5	1	3
	Lead Acetate	×	8	3	1
IV	Arsenic trioxide	×	4	4	0
	Arsenic trioxide	×	5	3	1
	Arsenic trioxide	×	8	3	1
V	None	None		3	3

TABLE 4.—CONTINUATION OF RAT INSECTICIDAL FEEDING INVESTIGATION USING NEW LOT OF 34 RATS PRODUCED FROM LITTER OF ORIGINAL STOCK. THE PARENTS OF THIS LOT WERE FED INSECTICIDES FOR A PERIOD OF ABOUT 175 DAYS BEFORE THE LITTERS MAKING UP THE LOT WERE BORN. THE LOT WAS FED FOR 378 DAYS

(First Generation Stock Produced from Original Lot of 54 Rats)

Division	Spray Chemicals		Dosage times tolerance	Males	Females
I	Lead Arsenate	×	4	1	3
	Lead Arsenate	×	5	2	2
	Lead Arsenate	×	8	2	2
II	Calcium Arsenate	×	4	0	0
	Calcium Arsenate	×	5	2	2
	Calcium Arsenate	×	8	2	2
III	Lead Acetate	×	4	3	1
	Lead Acetate	×	5	2	2
	Lead Acetate	×	8	0	1
IV	Lead Arsenate	×	200	2	2
V	None	None		2	0

DISCUSSION OF RESULTS
(Original Rat Stock)

Rats Receiving Lead Arsenate.—When the amount of lead arsenate was increased, the average gain in weight of the rats for the first 12-week period increased. For the remaining 12-week periods, the weight of the rats remained normal. The average gain per week was not influenced by the amount of arsenic fed. Furthermore, the weight of the rats was not changed by the arsenic fed for the entire feeding period.

Compared with the controls—rats which did not receive insecticides —those fed .04 grain of arsenic trioxide actually gained more during the first twelve weeks. This gain amounted to 2.35 grams more per week than the gain of the rats receiving no arsenic. At the end of sixteen weeks, those lots receiving .05 and .08 grain and those receiving no arsenic differed little, if any, in weight, activity or ability to produce young. In comparison with the controls, lots receiving .04 grain, did not show material difference in weight, activity and reproduction at the end of the same period.

Rats Receiving Calcium Arsenate.—During the first twelve weeks, the treated rats showed no material difference in gain, reproduction or activity when compared with the untreated lot. As the amount of calcium arsenate was increased, the average weekly increase in weight decreased. When fed .04 grain, the increase in weight was slightly greater than that of the controls. In the other lots the gain was less in a like comparison. The weekly gain (average) for the feeding period was very similar to that of the untreated rats.

Rats Receiving Soluble Lead Acetate.—Rats receiving soluble lead acetate in amounts as found in lead arsenate when .04, .05, and .08 grain of trioxide is present show that the gain is normal for the first twelve weeks. During the remaining periods of feeding, there was no correlation between amounts received and weekly average gain. When given .04 grain, increase in weight as measured by weekly gain was greater than that of control rats. The gains made by the other lots receiving .05 and .08 grain respectively, were similar to the gains of the controls; in fact, gain was similar in all lots, and slightly higher than untreated rats. In the same comparison, the average mortality was somewhat greater in lots receiving soluble lead acetate.

Rats Receiving Arsenic Trioxide.—There was no correlation between the gain in weight and the amount of arsenic trioxide fed. The gains made were very much like that of the controls. It was slightly more rapid, however, in the lot fed .04 grain than in the controls and slightly less in lots fed .05 and .08 grain. Gain based on weekly averages was slightly greater, but the deleterious effects were about 12 per cent greater when compared to controls. (See tables 7 and 8.)

First Generation of Offspring

Rats Receiving Lead Arsenate.—For the first 12-week period the weekly gain on an average for all lots was 2 grams less than the original or beginning lot for the same period. Compared with the controls, the males were 6.22 grams underweight. Comparing original control female rats with the first generation rats fed arsenic as lead arsenate, the controls gained 3.58 grams more per week. During the first 12-week period, as the amount of lead arsenate given increased, the rate of gain decreased. In the following 12-week periods no relation existed between amount of lead arsenate given and rate of gain in weight or growth.

Rats Receiving Calcium Arsenate and Lead Acetate.—The rats receiving calcium arsenate reacted in no way materially different from those receiving lead arsenate. Likewise, the rats receiving soluble lead acetate did not differ in reaction from those described in the beginning or original lots.

Rats Receiving Soluble Arsenic Trioxide.—As the dosage of arsenic trioxide was increased, the increase in weekly weights was not so great during the first and second 12-week periods. The average weight for male rats was 6.02 grams less than that of controls during the first 12-week period. The gain for both sexes was not as great as for controls.

The rats of this generation, shown in Table 4, which were fed arsenic trioxide, produced smaller litters by far than did the original stock. It was impossible to raise any of their young to four weeks of age. This inability to raise the young was attributed to the fact that the mothers failed to produce an adequate milk supply, some, in fact, failing entirely. Some of the females were apparently rendered sterile by continued feeding of the insecticide. As compared to controls, the litters of those producing young were fewer in number in every instance and the individuals were smaller in size.

Results From Feeding Large Dosages

Rats Fed 200 Times World Tolerance.—The results from one lot of four rats fed lead arsenate amounting to 200 times the world tolerance of .01 grain of arsenic trioxide per pound of fruit proved very interesting. The use of this excessive amount of arsenic was, first, to determine the assumed toxic effects of large dosages; and, second, to ascertain whether or not any of the effects of the insecticides are carried over from parent to offspring, or whether any weakness might by chance be inherited.

This lot of four rats was fed for 378 days. Gains over the controls for the first 12 to 16 weeks were comparable to those of other lots. In the five litters produced, they gave an average of five young per litter. This is important in that no other rats in this lot produced as many litters or as many total progeny. This may be explained partly by the fact that one male and one female were taken from untreated parents.

The other male and female were taken from parents fed lead arsenate eight times the tolerance for 119 days.

The weight and general appearance of the young were normal. In no case, however, were the mothers able to nourish the young. There were no losses among the original rats—a fact of special significance in view of the low legal tolerance. Acute toxic action seemed almost negligible. During the latter part of the feeding period, the eyes of the rats seemed to be more glassy and watery than those of the controls and the rats of other lots. Furthermore, when these rats were killed and the bodies dissected, the effects of both arsenic and lead were more striking and pronounced.

TABLE 5.—AFTER 71 WEEKS OF FEEDING THE ORIGINAL 54 RATS SHOWED THE FOLLOWING AS TO (1) NUMBER OF LITTERS BORN, (2) NUMBER IN RESPECTIVE LITTERS, AND (3) NUMBER OF PROGENY RAISED

Spray chemical fed	Times tolerance	No. of litters born	No. in respective litters	No. progeny raised
Lead arsenate	4	4	9, 8, 6, 6	7
Lead arsenate	5	3	10, 7, 8	22
Lead arsenate	8	4	11, 12, 9, 11	21
Calcium arsenate	4	4	8, 10, 7, 4	0*
Calcium arsenate	4	2	8, 8	5
Calcium arsenate	5	4	11, 8, 4, 5, 6, 8	10
Calcium arsenate	8	2	9, 7	16
Arsenic trioxide	4	all males	0	0
Arsenic trioxide	5	4	8, 8, 9, 4	15
Arsenic trioxide	8	3	11, 11, 9	28
Controls	0	4	9, 10, 8, 7	9
Controls	0	3	6, 10, 8	8

*Mother rat killed all but two of progeny.

TABLE 6.—A LOT OF 34 RATS CONSISTING OF THE OFFSPRING OF THE ORIGINAL LOT WHEN FED FOR 54 WEEKS GAVE THE FOLLOWING IN LITTERS BORN, NUMBER IN LITTERS, AND PROGENY RAISED

Spray chemical fed	Times tolerance	No. of litters born	No. in respective litters	No. of progeny raised
Lead arsenate	4	Female sterile	0	0
Lead arsenate	5	1	2	0
Lead arsenate	8	2	7, 4	0
Calcium arsenate	4	0	0	0
Calcium arsenate	5	0	0	0
Calcium arsenate	8	2	8, 7	0
Soluble lead	4	1	10	0
Soluble lead	5	0	0	0
Soluble lead	8	Female sterile	0	0
Arsenic trioxide	5	1	13	0
Lead arsenate	200	5	8, 5, 5, 2, 5	0

Twelve litters were produced from the lot of 34 rats. The young were underweight and weak. For the first two weeks after birth, they continued to die. Unfortunately, during the third week, brown rats killed all the remaining young. It is possible, therefore, that at least a few of the progeny of this first generation might have lived to maturity but for this accident.

TABLE 7.—AVERAGE GAIN—12 WEEK PERIODS—TO HEAVIEST WEIGHT—FOR LIFE—LOSS AND NO. WEEKS—ORIGINAL GENERATION

Tolerance	Average Gain per Week						Loss per wk. from heaviest weight	Gain per wk. for life	No. wks. to heaviest	No. wks. after heaviest	Length of life in wks.
	12 wks.	24 wks.	36 wks.	48 wks.	60 wks.	To heaviest					
La 4♂	18.79	5.84	1.25	1.38	−1.48	7.80	.44	4.17	51.0	18.0	69.0
La 4f	14.75	3.23	4.38	−3.12	1.25	8.86	2.26	4.04	32.0	22.0	54.0
La 5♂	15.38	6.67	1.54	.84	.42	5.60	.25	4.18	49.0	18.5	68.5
La 5f	9.38	3.96	.20	−.62	.62	6.00	.75	3.48	29.0	24.5	53.5
La 8♂[4]	9.92	4.88	.42	2.50	.83	10.22	1.50	6.02	21.0	20.0	41.0
La 8f	10.08	1.92	.12	.20	1.46	4.02	.57	2.05	37.0	28.5	65.5
Ca 4♂	16.38	5.04	.42	1.67	−.21	6.96	1.75	4.06	42.0	22.0	64.0
Ca 4f	9.92	1.79	1.25	.62	−.62	4.06	1.86	2.13	40.5	16.5	57.0
Ca 5♂	16.8	4.16	.61	1.04	−.21	6.37	1.81	2.91	49.5	21.5	71.0
Ca 5f	10.62	1.96	1.79	−.42	.84	3.36	.28	2.38	56.0	15.0	71.0
Ca 8♂	15.75	4.75	.97	1.46	0.0	4.22	1.47	4.01	54.0	10.0	64.0
Ca 8f	10.84	.92	.00	.25	0.0	4.90	.16	3.08	32.5	20.5	53.0
Pb 4♂	17.21	4.38	−.25	0.00	0.0	9.94	1.78	5.62	26.5	13.5	40.0
Pb 4f	11.84	.67	−.12	+1.04	0.0	7.13	2.95	2.08	27.0	27.0	54.0
Pb 5♂[1]	17.00	4.17	−.58	1.67	1.25	4.80	0.0	4.58	64.0	3.0	67.0
Pb 5f	12.64	.93	−.27	.57	.83	3.68	.53	2.31	47.0	20.0	67.0
Pb 8♂[2]	13.19	4.53	1.48	1.58	0.0	6.69	1.09	3.76	44.0	20.0	64.0
Pb 8f	11.25	2.25	.67	1.67	1.25	3.67	0.0	3.31	64.0	7.0	71.0
As_2O_5 4♂[3]	16.98	2.62	.12	1.26	2.08	8.48	.70	4.06	48.2	15.3	63.5
As_2O_5 5♂[2]	17.06	3.31	1.17	−1.39	1.81	6.50	.54	4.38	48.7	11.3	60.0
As_2O_5 5f[1]	11.75	−.42	1.67	.83	2.50	3.16	0.0	3.05	62.0	2.0	64.0
As_2O_5 8♂[2]	16.89	2.92	1.81	−.58	−1.25	6.96	2.22	3.85	43.7	20.3	64.0
As_2O_5 8f[1]	11.67	.33	0.0	1.67	0.0	3.13	0.0	2.56	46.0	18.0	64.0
Control	16.64	2.72	1.05	.31	.42	7.02	.65	3.45	42.3	25.4	67.7
Control f	12.20	1.03	.89	.14	.58	3.68	.26	2.31	51.3	19.7	71.0

(4) One rat died at eleven weeks; (1) one record; (2) three records; (3) four records.

After 12 weeks of arsenic feeding, the rats produced their first litter, which was an average of 8.2 young for each female. Furthermore, the same females after 25 weeks' feeding produced an average of 9 young in their second litter. For the same periods, the control rats produced 7.5 young in their first litter and 8 young in their second.

It was very clear that about the 25th week of feeding represented the highest or maximum growth and reproduction. If activity can be measured by careful daily observation, this period was also greatest for liveliness and agility. It is also significant that lead arsenate, arsenic trioxide, and lead acetate had similar effects in promoting activity and growth for this period.

After about the 23rd to the 25th week, the number of young in the litters decreased slowly. This was shown in the third litter when an average of 8 young was produced while in the fourth litter an average of only 6.5 was produced.

TABLE 8.—AVERAGE GAIN—12 WEEK PERIODS—TO HEAVIEST WEIGHT—FOR LIFE—LOSS NO. WEEKS—FIRST GENERATION OF OFFSPRING

Tolerance	Average Gain per Week 12 wks.	24 wks.	36 wks.	48 wks.	Gain per wk. to heaviest weight	Loss per wk. from heaviest weight	Gain per wk. for life	No. wks. to heaviest weight	No. wks. after heaviest weight	Length of life in wks.
La 4♂[1]	13.50	3.33	2.50	.42	8.26	0.0	7.81	29	3	32
La 4f[2]	10.98	1.80	.56	.27	6.18	.48	4.75	37	7	44
La 5♂	10.58	4.58	2.30	−1.46	5.97	6.06	4.60	38	11	49
La 5f	7.29	1.86	1.79	.83	3.52	0.0	3.60	42	10	52
La 8♂	11.62	3.92	2.29	.42	5.65	.31	4.22	41	13	54
La 8f	7.58	2.12	.62	.42	4.22	.72	2.56	37	17	54
Ca 5♂	9.83	4.38	0.0	0.0	6.78	1.45	3.22	38	12	50
Ca 5f	9.00	3.38	.62	−1.67	4.60	----	3.86	36	18	54
Ca 8♂	13.46	4.50	.42	−2.08	7.16	1.88	3.53	33.5	18.5	52
Ca 8f	9.55	2.71	1.25	−.41	6.01	.09	2.99	27.5	26.5	54
Pb 4♂[2]	16.58	5.58	.62	----	8.32	1.45	5.87	34.3	10.7	45
Pb 4f[1]	17.50	6.25	.83	----	9.36	----	6.56	22	23	45
Pb 5♂	11.04	4.58	3.33	1.25	4.94	----	4.85	46	4	50
Pb 5♂	8.30	2.79	1.25	0.0	4.84	----	2.97	31	19	50
As_2O_5 5♂	13.04	3.96	.83	.84	7.28	----	4.72	31	17	48
As_2O_5 5f	10.86	1.17	.20	.42	6.30	----	3.32	36.5	11.5	48
As_2O_5 8♂[1]	10.83	1.67	----	----	7.37	----	5.64	19	6	25
As_2O_5 8f[1]	----	----	----	----	8.75	2.75	1.17	4	7	11
Control ♂	18.12	5.20	0.0	0.0	9.79	----	6.35	24	13	37
La 200♂	15.75	3.58	3.33	−.21	5.38	.25	4.24	44	10	54
La 200f	12.96	2.04	2.5	−.84	6.10	1.27	2.85	30	24	54

(1) One Record; (2) 3 records.

POST MORTEM EXAMINATION

At the close of the feeding experiment, every rat was killed and a post mortem made. The rats which received arsenic as lead arsenate or as calcium arsenate showed similar symptoms. These symptoms were: (a) watery eyes (glassy), (b) lungs inflamed, and (c) bad diarrhea. Rats receiving lead in lead arsenate or as soluble lead acetate showed similar symptoms which were: (a) discolored liver, (b) mesentery inflamed with blood vessels enlarged, (c) congestion or inflammation of the intestines, (d) dark discoloration of toe nails near base, and (e) discoloration of the flesh at the base of the teeth. Post mortem of control rats showed none of the symptoms given above.

MORTALITY

Of the 54 original rats, 48 received the treatment and six were fed a normal ration with no treatment. The feeding period was for 497 days.

Seventeen of the 48 receiving treatment died, which gave a mortality of 35.4 per cent. About 90 per cent of the deaths occurred after the long feeding period of 400 days had been reached. None of the control rats died.

Thirty-four rats of the first generation were fed for 378 days. They were given the same treatment and rations as the original lot. Of this number five died, which constituted a loss of 14.7 per cent. This lower mortality, compared with the original lot, is due, no doubt, to the shorter feeding period. For example, a feeding period of the offspring generation rats was 378 days, while that of the original lot was for 497 days.

LITERATURE CITED

1. O'Kane, Hadley, and Osgood. *Arsenical Residues After Spraying*. N. H. Agr. Exp. Sta. Bul. 183, 1917.

2. Streeter, Chapman, Harman and Pearce. *Spray and Other Deposits on Fruit*. N. Y. (Geneva) State Agr. Exp. Sta. Bul. No. 611, Apr. 1932.

3. Talbert, T. J. and Tayloe, W. L. *The Spray Residue Problem*, Proc. Amer. Soc. Hort. Sci. 1930.

ACKNOWLEDGMENT

The writers wish to acknowledge the valuable suggestions, cooperation, and assistance given from time to time by Dr. C. W. Greene, Professor of Physiology and Pharmacology, School of Medicine, University of Missouri.

The cooperation and assistance given by C. H. Manville, Commissioner, State of Missouri Food and Drug Department, has been very valuable.

The excellent assistance of Mr. Carl Lewis, regular attendant, a student of the College of Agriculture, University of Missouri, makes the report possible.