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On the penetration of certain arsenical compounds into the body of the American cockroach, Periplaneta americana (L)

Leon Conrad Glover

Iowa State College

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UMI®
ON THE PENETRATION OF CERTAIN ARSENICAL COMPOUNDS INTO THE BODY
OF THE AMERICAN COCKROACH, Periplaneta americana (L.)

by

Leon Conrad Clever

A Thesis submitted to the Graduate Faculty
for the degree of
DOCTOR OF PHILOSOPHY
Major Subject Entomology

Approved

Signature was redacted for privacy.

In charge of Major work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1936
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INTRODUCTION

Arsenical compounds have been used as insecticides for many years, yet little attention has been given to their effect as contact poisons. Recent workers have demonstrated the ability of toxicants to pass through the integument of insects in both the liquid and gaseous phase. Other work seems to show that arsenicals may act as contact poisons by entering the breathing system or by penetrating through the insect's integument in sufficient amounts to bring about death.

This work was undertaken to determine the nature of the penetration of certain arsénical compounds through the integument when applied in the form of a dry powder and to learn to what extent such compounds became distributed in the insect body after penetration has taken place.

A comparison was also made of the penetration and distribution of arsenious oxide and sodium arsenite.
HISTORICAL

Until 1923 the use of arsenicals as toxicants for insects had been largely as stomach poisons. Mally (1923) found that sodium arsenite killed insects rather quickly when applied as a dust to the antennae of grasshoppers. He had been testing the action of sodium arsenite as a stomach poison and states it acted as a contact poison when applied to the antennae, apparently without ingestion.

As early as 1909 sodium arsenite was used as a contact poison. Cousins (1924) describes the use of "arsenic" in a paraffin-naphthalene emulsion as a spray for cattle. In a dip used for the same purpose two pounds of 80.0 per cent sodium arsenite and three pounds of paraffin-naphthalene emulsion in 100 gallons of water made an efficient insecticide for ticks on cattle.

The use of sodium arsenite against grasshoppers proved to be so effective that it was used in both the Sudan and the Ukraine. The tremendous numbers of these insects occurring in a territory inhabited by few people, and those natives, enabled experimenters to use this poison where it would have been impossible in other parts of the world.

Williams (1924) tested a spray on grasshoppers which contained three ounces of sodium arsenite powder to four gallons of water.

Nikolski (1924) describes a series of experiments made in South Africa, in which he applied sodium arsenite as a
dust. This was successful in controlling grasshoppers.

Soon after Mally had published the results of his work, Russian investigators started a series of researches with arsenicals as contact poisons. This had been suggested to them by Uvarov (1924) who felt that the addition to the arsenicals of some neutral powder such as lime might reduce the cost without affecting results. The Russian workers conducted experiments in the laboratory as well as in the field with the view of obtaining more exact data which might enable them to cut the cost of materials employed against the grasshoppers.

Vuichelesskaya (1926), studying sodium arsenite as a contact insecticide, found a direct increase in the toxicity of sodium arsenite as its \( \text{As}_2\text{O}_3 \) content increased. The lethal dose to roaches of sodium arsenite containing 28.15 per cent \( \text{As}_2\text{O}_3 \) was 0.118 milligram and for sodium arsenite containing 42.86 was 0.245 milligram. One roach survived which contained 1.05 milligrams of \( \text{As}_2\text{O}_3 \). This investigator suggests that in cases of very slow poisoning the arsenic may accumulate in the body of the insect.

Granovsky (1926) states that the work of Mally (1923) led to a series of experiments by Sovdarg. Sovdarg considered that his studies demonstrated the penetration of arsenic "through the chitin" of insects. It is stated that the poison has an action on the nervous system. Insects would die when only their bodies were dusted. In this case
the insects had no opportunity to lick the appendages within reach of their mouths. It is stated further that even on relatively clear, hot days there was enough "moisture" present on the insect body to enable the dust to adhere and bring about death. As a spray sodium arsenite killed even before the water of the spray had evaporated from the body. This rapid toxic action is considered to be due to the rapid penetration of poison "through the chitin". Sevdarg states also that a high humidity is an important factor for the rapid physiological action of arsenical poisons.

In observing grasshoppers that had crawled over a poison bait containing sodium arsenite, Granovsky (1926) found that the ventral surface of the posterior part of the thorax and the anterior part of the abdomen seemed "burnt" and disintegrated. This he ascribes to the corrosive or caustic action of the poison in the bait. Other parts of the surface of the body seemed unaffected. Granovsky states that Bolodgrev in commenting on the work of Mally (1923) suggests that possibly the insects obtained the poison from their antennae and that it acted as a stomach poison.

Both Smith (1926) and Parfentjev (1926) report increased toxicity of arsenicals when sulphur compounds were added. Smith worked with lead arsenate dust and compared it to the spray while Parfentjev tested calcium arsenate as a dust, both with and without lime and sulphur. No statement is made as to what part, if any, of the toxic action is due to the toxicant's
being taken in through the insect's mouth.

Strawiński (1926) proposes the use of arsenical candles. A dense cloud of smoke is given off when the candles burn. This envelops the trees and settles on the foliage in the form of As₂O₃. Young larvae of *Cheimatobia frumata* L. were killed 11 to 16 hours after contact with the smoke. Aphids and Psyllids were killed more quickly, he states.

Parfentjev (1926) found that roaches which were fed bread containing 2.5 per cent calcium arsenate (died 2-4 days) held an average of 0.3-0.5 milligram of arsenic pentoxide within the body.

Using sodium arsenite as a spray Ordoñez (1926) killed the larvae of *Galerucella luteola* (now *xanthomelaena*) Schrank in 36 to 48 hours.

Zakharov (1927) worked with sodium arsenite as a contact poison and states that its contact effects were increased by the humidity of the air (dew).

Parfentjev (1928) states that sodium arsenite is more effective as a contact poison when in solution. This investigator shows that a water solution of sodium arsenite collects in drops on the integument of a cockroach. By adding soap an even coating of the liquid was secured, but under laboratory conditions this difference in coverage had little influence on the final effect of the poison. In the field, grasshoppers hit with the spray succumbed without moving from the spot. No statement is made as to how much
of the toxic action is due to penetration through the spiracles into the tracheal system.

In recommending a toxicant for grasshoppers Harrison (1929) suggests a spray containing five ounces of sodium arsenite to four gallons of water.

Shotwell (1930) cites an instance in northern Montana of the use of sodium arsenite in a "one to sixty-four" solution.

King and Ruttledge (1932) carried out a series of experiments with dusts and found that sodium arsenite (240 mesh) brought about death when adults of *Locusta migratoria*oides *Sch.* and *Frm.* were allowed to flutter in a cloud of it for twenty seconds. Precautions were taken to keep the dust from the digestive tract. The tracheae were found to contain appreciable quantities of arsenic but the authors state that there was no evidence that the arsenic had penetrated the integument of the grasshoppers. The speed of action apparently depended upon the solubility of the arsenic salt.

Golding (1932) made a study of the effects of poisons on grasshoppers. Tests were made on lots of 500 adults sprayed with a solution of five ounces of sodium arsenite in four gallons of water. Such a lot of 500 adult grasshoppers contained a total of two grains of arsenic.

Pemberton (1932) in controlling the nutgrass armyworm prefers to dust while the larvae are still on the plants. This worker recommends the addition of five pounds of white arsenic to thirty pounds of finely divided raw rock-phosphate.
No statement is made as to what part of the toxic action is due to the contact effect.

Percival and Potter (1932) found that more accurate results could be obtained, when using the Gutzzeit method for the determination of arsenic, by weighing the sulphuric acid used in each determination.

Jack (1933a, 1933b) recommends the use of sodium arsenite solution as a spray for grasshoppers. By spraying the grass immediately in front of a moving swarm the hoppers are killed by being wetted in passing through the sprayed strip. It is his belief that baits and dusts are often impracticable because of tall grass and too wet weather.
EXPERIMENTAL

Materials

Biological. The insects in this investigation were adults of the American cockroach, *Periplaneta americana* (L). Part of the roaches were reared in a constant temperature cabinet held at 27°C and 90 per cent humidity - 5 per cent. Eggs and successive stages of nymphs were confined in separate glass jars. Adults were segregated as soon as they moulted from the last nymphal stage. All roaches were fed a diet consisting of 200 grams graham flour, 20 grams olive oil and one gram Harris' Brewer's yeast. Water was kept before the insect culture by means of a small fountain.

A second group of roaches was trapped in a cotton mill in Dover, New Hampshire. These were held in the constant temperature cabinet for at least two weeks before being used. Other roaches came from Alabama. These were handled in a manner similar to the roaches trapped in Dover.

Chemical. The toxic chemical compounds used were arsienious oxide, As$_2$O$_3$, special anhydride powder and sodium arsenite, purified powder, 85.1 per cent As$_2$O$_3$. These and the following were from the J. T. Baker Chemical Company.

The chemical compounds used in the digestion of the biological material were nitric acid HNO$_3$, Sp. gr. 1.415-1.42, 68-70 per cent, (meets A. C. S. Standard); sulphuric acid, H$_2$SO$_4$ at least 94 per cent, special, sp. gr. 1.835-1.84, low
in nitrogen and arsenic, (meets A.C.S. Standard); and ammonium oxalate \((\text{NH}_4)_2 \text{C}_2\text{O}_4 \text{H}_2\text{O}\), purified crystal.

Other chemical compounds used in the Gutzeit method for determining arsenic were lead acetate, U.S.P. powder; stannous chloride \(\text{SnCl}_2 \cdot 2\text{H}_2\text{O}\), purified, crystal; mercuric bromide \(\text{HgBr}_2\), C.P. Baker’s analyzed crystals; sodium hydroxide \(\text{NaOH}\), C. P. Baker’s analyzed pellets; hydrochloric acid \(\text{HCl}\), 35-37 per cent, special arsenic free; and zinc metal, 20 mesh, special, low in arsenic, granular (meets A.C.S. Standard).

The chemical compounds used in the Gutzeit method were employed as described under that method in A. O. A. C. (1930) unless stated otherwise under methods.
Methods of Procedure

The powder, either arsenious oxide or sodium arsenite was applied to the roach by means of a beeswax cell. This cell was placed upon the dorsal surface of the metathorax in the following manner. The roach was first prepared for receiving the cell by cutting away a part of the wings in order to expose the full width of the dorsal metathorax. The part of the front wing from the anal sulcus in the center of the wing to the inner margin and about one-third of the posterior part of the hindwing, including the folded anal portion, were removed.

The cell was applied by holding it in position with the thumb and index finger then sealing it in place by melting a part of the side walls with a heated dissecting needle which had been bent at right angles about one-fourth inch from the end. Care must be taken that the entire lower surface of the cell wall is made fast, including the inner margin of the wall, for otherwise particles of the toxic material may work out of the cell and into intersegmental spaces. The melted wax must of necessity come from the outer part of the wall since melting of the inner wall allows the beeswax to run across the bottom of the cell thus reducing the area available for penetration.

Small amounts of the toxic material are transferred to the cell upon the tip of a small spear scalpel blade. It is best that the toxic material be no greater in amount than will cover a space on the scalpel blade equal to the depth of the
cell. With this precaution it is possible to lower the blade tip into the cell, turn the blade over, tap it gently with the index finger, thus depositing the toxic material in the cell without spilling any outside. After the required amount has been placed in the cell it is spread out in order to cover the entire area through which penetration may take place.

As soon as the toxic material is in the cell a cap is placed in position and sealed on with a hot needle. Care should be taken at this point not to heat the wax of the cap so hot that it will melt and run into the cell because if this happens the wax displaces the toxicant, adheres to the integument and reduces the area available for penetration.

The insect is then washed thoroughly under a stream of distilled water to eliminate any contamination which might come from unseen particles of the toxicant in the air. Finally it is placed in an ordinary drinking glass which has been fitted with a raised screen bottom. This bottom prevents the roach from eating voided fecal matter, which might contain arsenic.

As no attempt was made to allow all of the toxic material to penetrate through the integument the exact weight of the material applied was not recorded. Experiments were carried out in which in one group the amount of toxicant was tripled and in another the area of the cell was doubled. The results of these experiments are given in Table II.

In the preliminary work the roaches were kept under lab-
oratory conditions. Food and water were kept before each roach. In later experiments the roaches were kept under the same conditions as that of the stock culture. Neither food nor water was given to the treated insects because early experiments had shown that a roach with water in its digestive tract was apt to regurgitate the liquid contents of the tract thus affecting final results. Roaches which have not had access to water seldom do this.

Treatment was varied in time from twenty-four hours to seven days. At the end of the period of treatment the roaches were killed with chloroform and the cell removed at once. In removing the cell the roach was held with the dorsal surface down and the cell carefully pried off. Thus any loose dry powder fell back into the cell and did not come into contact with the insect. The damp powder which adheres to the integument was scraped away by means of a small scalpel while the roach was still held in an inverted position. In roaches treated with sodium arsenite the small noticeable amount of liquid present under the cell was absorbed with a small piece of filter paper after the damp powder had been removed. The roach was then washed first under a stream of tap water then in a stream of distilled water in order to remove any trace of toxic material which might remain.

Check roaches were treated in the same way as roaches used for penetration studies with the exception that the cell containing the arsenic was removed as soon as possible after
it had been sealed in position.

Entire roaches were placed directly into tared Kjeldahl flasks together with four milliliters of sulphuric acid and five milliliters of nitric acid. Digestion could be carried on with less bumping when the sulphuric acid was allowed to stand with the roach overnight before the nitric acid was added. After digestion enough sulphuric acid was added, Percival and Potter (1932), to make a total weight of nine grams + 0.01 gram.

When the roaches were to be dissected the legs, wings and head were removed in that order then the roach was sealed into beeswax with its ventral surface downward. The dorsal surface was removed by cutting around the lateral conjunctivae between the tergites and sternites. All adhering tissues were scraped from the surface of the dorsal sclerites as they were removed. After the removal of the dorsal surface the digestive tract was withdrawn as one unit, care being taken to clean off adhering fat body and the Malpighian tubes. The genital organs were removed next followed by the central nervous system. The portion of this system which was used consisted of the abdominal and thoracic ganglia, the commissures and a part of the larger nerves. Finally the fat body and then the thoracic muscles were removed. As preliminary work showed only traces of arsenic in the Malpighian tubes and fat body they were removed with the fat body. Parts to be digested were handled in tared Kjeldahl flasks in a manner similar to whole roaches. Tared
flasks were employed because to obtain uniform stains by means of the Gutzzeit method it was necessary to have the same amount of acid in each determination. By placing the whole roach or parts of roaches in tared flasks it was possible to make the acid up to a standard weight after digestion had been accomplished.

The amount of arsenic present was determined by a modified Gutzzeit method. The Gutzzeit generator was modified somewhat, as follows: Instead of the usual two pieces of tubing held together by a cork the tubing was made as a unit. Paraffined corks were employed in the place of rubber as a precaution against contamination.

After the digested material was brought to a standard weight it was transferred to a 25 milliliter volumetric flask. The Kjeldahl flask was rinsed at least four times with distilled water after the transfer had been made.

The use of aliquots was unnecessary, the whole sample being placed in the generator. Since, with the dissected parts of roaches only minute amounts of arsenic could be expected, the concentration of the mercuric bromide solution for the paper strips was reduced to 1.5 per cent. For whole roaches the concentration was varied from four per cent to nine per cent. The initial charge in the generator consisted of three grams of 20 mesh granulated zinc, 2.5 milliliters of distilled water, and one milliliter of hydrochloric acid - stannous chloride solution.
Immediately after the digested material was added to the Gutzeit generator bottle it was placed for one and one-half hours in a constant temperature bath held at 25°C ± 0.2°. One blank was run with each lot.

The sensitized paper strips were dried 15 minutes and used at once. After the arsine had been evolved the strips were removed and dipped in paraffin which was held at a temperature just above melting point. Then the strips were observed under a binocular microscope and the extent of the stain on each side marked, so that an accurate measurement could be made. The figures given in the tables were obtained by taking an average of the two sides of the stained sensitized strip to compare with a standard graph. Each time that a series of determinations is made on solutions containing an unknown amount of arsenic, a series of determinations is made employing a range of dilutions of standard arsenic solution made up from arsenious oxide, As₂O₃, that will exceed any amount which may be found in the unknown. A standard graph is constructed by plotting on cross-sectioned paper the averages of the measurements from the standard arsenic solutions. Thus all results given in tables are read directly as arsenic in milligrams of As₂O₃.
RESULTS

All the roaches used in these experiments could not be obtained from the same source. Many were available which had been reared under constant conditions. Others had to be secured from other sources. Table I gives the results of a test designed to find if it was necessary to use roaches reared under constant conditions in order to obtain uniform results. The average concentration of arsenious oxide present in the bodies of adult roaches, reared under constant conditions, after having been treated for 72 hours was 0.008 mg./g. body weight; after 120 hours 0.039 mg./g. body weight; and after 168 hours 0.022 mg./g. body weight. The average concentration of arsenious oxide present in the bodies of adult roaches, trapped in a cotton mill, after having been treated for 72 hours was 0.011 mg./g. body weight; after 120 hours 0.010 mg./g. body weight; and after 168 hours 0.004 mg./g. body weight. (Table I).

The average concentration of arsenious oxide present in the bodies of roaches when the area of the application cell had been doubled was 0.016 mg./g. body weight; that of roaches treated with the standard cell was 0.006 mg./g. body weight. (Table II).

In experiments where the amount of arsenious oxide powder was varied, the average amount of arsenic recovered as arsenious oxide was 0.006 mg./g. body weight when one one-hundredth gram was used and 0.007 mg./g. body weight when three one-hun-
dredths gram was used. (Table II).

The average concentration of arsenious oxide in the bodies of adult roaches treated with anhydrous arsenious oxide for 72 hours was 0.010 mg./g. body weight; in 120 hours 0.025 mg./g. body weight; and in 168 hours 0.021 mg./g. body weight. (Table III Chart I). Check roaches contained on an average 0.001 mg./g. body weight.
Table I

Penetration of Arsenious oxide into Adult Periplaneta americana (L)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exposure time</th>
<th>Number of roaches</th>
<th>As$_2$O$_3$ Recovered</th>
<th>As$_2$O$_3$ Recovered</th>
<th>Mg$_2$/G. Body Weight</th>
<th>Mg$_2$/G. Body Weight</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenious oxide</td>
<td>72</td>
<td>30</td>
<td>0.003-0.019</td>
<td>0.008</td>
<td>0.001-0.071</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>30</td>
<td>0.008-0.115</td>
<td>0.039</td>
<td>0.002-0.020</td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>168</td>
<td>30</td>
<td>0.003-0.017</td>
<td>0.022</td>
<td>0.001-0.007</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table II

Concentrations of Arsenious oxide in Adults of Periplaneta americana (L) after Varying the Area Exposed and after Using Different Amounts of the Arsenious oxide in the Confining Cell

<table>
<thead>
<tr>
<th>:Num-</th>
<th>Cell area doubled</th>
<th>1 gm. Oxide in Re-</th>
<th>3 gms. Oxide in Regular Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>:Expo-sber</td>
<td>As₂O₃ Recovered</td>
<td>100 gular Cell</td>
<td>100</td>
</tr>
<tr>
<td>:sure :deter-</td>
<td>Mg₂/G.</td>
<td>As₂O₃ Recovered</td>
<td>As₂O₃ Recovered</td>
</tr>
<tr>
<td>:time :mina-</td>
<td></td>
<td>Mg₂/G.</td>
<td></td>
</tr>
<tr>
<td>:hours :tions</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 : 30</td>
<td>0.001-0.049 : 0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 : 30</td>
<td>0.001-0.043 : 0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 : 30</td>
<td>0.001-0.017 : 0.007</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In experiments in which various parts and tissues of roaches were tested arsenious oxide was recovered in greatest amounts from parts located nearest the point of application and from the digestive tract. The amount in the integument increased slightly from 24 to 72 hours. In the thoracic muscle the arsenic recovered increased materially from 24 to 48 hours and remained practically constant from 48 to 72 hours. In the legs and reproductive organs a slight increase was noted from 24 to 48 hours and a materially larger increase from 48 to 72 hours. The amount of arsenic recovered from the digestive tract increased steadily from 24 to 72 hours. Only very small amounts were recovered from the fat body and central nervous system. (Table IV Chart II).

The average concentration of arsenic, recorded in milligrams of As₂O₃, recovered from whole roaches treated with sodium arsenite powder for 72 hours was 0.073 mg/g. body weight; in 120 hours, 0.103 mg/g. body weight; and in 168 hours, 0.162 mg/g. body weight. (Table III Chart I).

In experiments in which various parts and tissues from roaches treated with sodium arsenites were tested the largest amounts were recovered as in the case of arsenious oxide, from parts nearest the point of application and from the digestive tract. The amount recovered from the integument, legs, wings and digestive tract showed a definite increase from 24 to 72 hours. The amount recovered in the central nervous system and reproductive organs showed no appreciable increase until 72
hours. With the thoracic muscle and fat body the amount recovered was variable over the whole period. The 48 hour figure for thoracic muscle is apparently in error. The 72 hour amount was nearly three times that of the 24 hour period. Parts and tissues from untreated roaches were as follows: Integument 0.0006 mg./g. tissue, wings 0.0003 mg./g. tissue, legs, digestive tract, fat body, reproductive organs, central nervous system and thoracic muscle, trace.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Arsenic oxide</th>
<th>Sodium oxide</th>
<th>Arsenic</th>
<th>Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>166</td>
<td>20</td>
<td>0.039-0.510</td>
<td>0.152</td>
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<td>180</td>
<td>20</td>
<td>0.054-0.16</td>
<td>0.105</td>
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<tr>
<td>75</td>
<td>20</td>
<td>0.01-0.18</td>
<td>0.075</td>
<td></td>
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<tr>
<td>166</td>
<td>20</td>
<td>0.001-0.173</td>
<td>0.021</td>
<td></td>
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<tr>
<td>120</td>
<td>20</td>
<td>0.005-0.015</td>
<td>0.056</td>
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<tr>
<td>75</td>
<td>20</td>
<td>0.002-0.014</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

Table III

*Note: The table represents the content of arsenic oxide and sodium oxide in various compounds.*
Chart I. Concentration of Arsenous Oxide and Sodium Arsenite in Adult *Periplaneta americana* (L.)
CHART I.

TIME IN HOURS

CONCN. \( \text{AS}_2\text{O}_3 \) MG/G.

- SODIUM ARSENITE
- ARSENIOUS OXIDE

80. 100. 120. 140. 160.
Table IV--Distribution of Arsenic in Tissues of
Periplaneta americana (L.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity As₂O₃ recovered from parts and tissues in mg./g.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arsenious oxide</td>
</tr>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Time in hours</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>Integument</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
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<tr>
<td>Mean</td>
<td></td>
</tr>
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<td>Legs</td>
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Chart II. Distribution of arsenic in various body parts and tissues of *Periplaneta americana* (L.)
INTEGUMENT

TIME IN HOURS
CHART II

CONCENTRATION AS$_2$O$_3$ MG./G. TISSUE

LEGS

TIME IN HOURS AS$_2$O$_3$ =

CHART II NA ARSENITE=
DIGESTIVE TRACT

CHART II

REPRODUCTIVE ORGANS

CHART II

AS$_2$O$_3$ = ---
NA ARSENITE=---
FAT BODY

CENTRAL NERVOUS SYSTEM

CHART II
Discussion of Results

Arsenious oxide and sodium arsenite are arsenical compounds which vary widely in their solubility. Arsenious oxide is slightly soluble in cold water while sodium arsenite is very soluble.

These compounds have been compared with respect to their penetration through the integument of the dorsal metathoracic segment of the American roach. Factors which could affect penetration have been studied. The distribution of these compounds in the tissues is discussed. Finally the arsenic content of the voided feces was studied to determine whether the elimination of the arsenic was affecting the results of penetration.

Penetration. A study of the results obtained from a comparison of roaches reared under constant conditions and those trapped in a cotton mill showed that less arsenic was recovered from the roaches trapped in a cotton mill. (Table I). A statistical analysis shows that there is no significant difference between the means of the 24, 48 and 72 hour results. (Fisher's t table 1932).

Granevsky (1926) commenting on work done by Sovdarg states that Sovdarg considered his studies demonstrated the penetration of arsenic "through the chitin" of insects. In an earlier work Mally (1923) working with sodium arsenite as a stomach poison noticed it acted as a contact poison apparently without ingestion. Later King and Puttleedge (1932) carried out a ser-
ies of experiments which led them to believe sodium arsenite would not penetrate an insect's integument. The application cell described in the present work was designed by the author with the idea of clearing up the question of penetration through the integument. By placing the toxicant in a cell which had been sealed to the insect's metathorax and by sealing over the cell the possibilities of penetration or entry through spiracles, anal opening or mouth are eliminated. The results given in Table III show that penetration does take place. That roaches treated with arsenious oxide may eliminate the arsenic as fast as it is taken into the body is shown in Table III. The average amount of arsenic recovered is slightly less after 168 hours than after 120 hours. Feces tested for arsenic content contained an average of 0.002 mg. per roach after 72 hour treatment with arsenious oxide.

While it was logical to assume that an increase in the area to which the arsenical compound was applied would allow increased amounts of the toxicants to penetrate, an experiment was designed to obtain evidence in this regard. The results given in Table II show that approximately three times as much arsenic is recovered when the cell area is doubled. This was to be expected, for an increased area increases the chances for a toxicant to penetrate.

The amount of liquid on the insect integument was always greater when sodium arsenite was used. The arsenious oxide was damp and adhered to the integument, but there was never excess liquid present. When sodium arsenite was used increasing amounts
of liquid were present with increased time. When the penetration period was seven days practically all of the powder was dissolved.

Arsenious oxide is very sparingly soluble in water. When the oxide is boiled in water for an hour 2.5 parts of the oxide are retained by the water. Arsenious oxide is increasingly soluble in alkaline solutions due to the formation of soluble arsenites.

Sodium arsenite is readily soluble in water. This may account for the larger amount of liquid present in the application cell when sodium arsenite was used.

Assuming that penetration takes place only after the powder applied has become dissolved in the liquid present on the insect integument, no record need be kept of the amount of the powder placed in the cell as long as there was a sufficient quantity to last through the application period. Results of an experiment given in Table II bear out this assumption. When the amount of powder was tripled there was no significant difference in the amount of arsenic recovered after a penetration period of 72 hours.

In another experiment designed to learn the effect of variables the amount of arsenious oxide was varied. In one set of roaches one one-hundredth gram was placed in the cell and in another three one-hundredth gram. The results 0.006 mg/g. body weight for the roaches treated with one one-hundredth gram and 0.007 mg/g. body weight for those treated with three one-hundredths grams are not significantly different. When the whole
area of the bottom of the cell is covered the places where the toxicant can penetrate are all taken up. Excess powder does not become dampened or dissolved by the liquid on the insect integument. The chances of increased penetration are thus lessened, assuming the powder must be dissolved by the liquid on the integument before it can penetrate.

When a series of penetration studies were made, with arsenious oxide in the one case and with sodium arsenite in the other, the results show that sodium arsenite penetrates nearly seven times faster than arsenious oxide. (Table III Chart I). This fact is evidenced by the presence of more liquid in the cell at the end of the penetration period. Apparently sodium arsenite, solubility in water discussed above, is much more soluble in this liquid than is arsenious oxide.

**Distribution in the Tissues.**
A comparison of the distribution of arsenious oxide and sodium arsenite in various parts and tissues is given in Table IV and Chart II.

Sovdarg states that sodium arsenite has an action on the nervous system. This toxicant has a definite caustic effect. Very often the integument and underlying tissues which had been exposed to sodium arsenite were brown and disintegrated. The writer noticed no evidences of paralysis. Sometimes reaches could not move their legs and wings but these were found to have the thoracic muscles brown and partly disintegrated from the action of the sodium arsenite.

Two facts stand out in the distribution studies, viz.,
the greater proportion of arsenic recovered from the body of
the roach is found either in tissues or parts in close proximity
to the point of entrance, or in the digestive tract. From this
it seems that distribution takes place by diffusion through the
tissues rather than by transportation in the blood; that is, the
blood acts as a passive receiver and not as the agent responsible
for distribution. Further the application of the arsenic is
directly over the dorsal vessel which carries the blood directly
to the head. In previous work by the writer O’Kane and Glover
(1935) only traces of arsenic were found in the roach’s head.
This, together with the fact that little blood is present, and
distribution to all tissues takes place only after rather high
concentrations of arsenic are built up in the roach, seems to
substantiate the theory that distribution in the body is by
means of diffusion.

In the case of both arsenious oxide and sodium arsenite the
amount of arsenic recovered increases steadily throughout the
penetration period. (Table IV Chart II). This and the fact that
.002 milligram of arsenic per roach was recovered from the feces,
seem to point to the elimination of arsenic by the digestive tract.
The digestive tract acting as a means of eliminating arsenic may
receive the arsenic directly from the thoracic muscles.

When arsenious oxide is used as a toxicant only traces or
small amounts are recovered in the reproductive organs, the
central nervous system and the fat body. Apparently this is
due to the fact that arsenious oxide is slowly soluble and suf-
icient concentration has not been built up in the body to allow for diffusion to all parts. When sodium arsenite was used, increasing amounts with time are found in the tissues mentioned above. This is especially true with the central nervous system. The amount of arsenic recovered from this system after treatment with sodium arsenite increases rapidly after 48 hours, whereas the amount recovered after treatment with arsenious oxide is little more than a trace for the entire period. (Table IV Chart II).
SUMMARY AND CONCLUSIONS.

1. The American cockroach, *Periplaneta americana* (L.) was treated with anhydrous arsenious oxide and with sodium arsenite powder. Application was made by sealing a wax cell, containing the toxicant in the form of a dry powder, on the dorsal metathorax. Entire roaches as well as parts and tissues were tested by means of the Gutzeit method to determine the amount of arsenic recoverable. Results were recorded by means of paper strips sensitized with MgBr₂ and measured in milligrams of As₂O₃.

2. Experiments were designed to determine the effect on penetration of increased area available for penetration and to determine the effect of varying amounts of the powdered toxicant.

3. That arsenious oxide and sodium arsenite penetrate the dorsal metathoracic sclerite is demonstrated.

4. The concentrations in the bodies of roaches treated with anhydrous arsenious oxide were as follows: 72 hours 0.010 mg./g. body weight, 120 hours 0.025 mg./g. body weight and 168 hours 0.021 mg./g. body weight. When roaches were treated with sodium arsenite powder the amounts recovered were as follows: 72 hours 0.073 mg./g. body weight, 120 hours 0.103 mg./g. body weight and 168 hours 0.162 mg./g. body weight.

5. The ventral nerve cord does not take up appreciable amounts of arsenic when applied in the form of arsenious oxide. When arsenic is applied as sodium arsenite the central nervous system takes up increasing amounts with increased time.
of application.

6. With the exception of the digestive tract the thoracic muscle contained the largest amount of arsenic when applied either in the form of arsenious oxide or as sodium arsenite.

7. Arsenic is eliminated to a considerable extent by means of the digestive tract.

8. When higher concentrations of arsenic are built up in the body of the roach the arsenic penetrates to all of the parts and tissues tested. When a less soluble compound such as arsenious oxide is used the distribution of the arsenic is practically limited to the digestive tract and to parts and tissues near the point of application.

9. Penetration of the sodium arsenite and arsenious oxide when applied in the powdered form, apparently takes place only after the powder has dissolved in a fluid present on the integument of the roach.

10. The average weight of tissues and parts was as follows:
    - integument 0.235 gram
    - legs 0.228 gram
    - wings 0.016 gram
    - digestive tract 0.079 gram
    - thoracic muscle 0.090 gram
    - fat body 0.067 gram
    - reproductive organs 0.042 gram
    - central nervous system 0.005 gram.
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VITA

I, Leon Conrad Glover, son of Rachel Cora Holt and Hiram A. Glover, was born November 10, 1900 in Amherst, New Hampshire. My grade school work was completed in 1913 in Hollie district school and my secondary school training in Amherst (N.H.) High School in 1917. Until September 1919 I served as an association tester in Vermont. I was graduated in the General Agriculture course from the University of New Hampshire in 1923. Following graduation I spent four years as a dairy herdsman in Cody, Wyoming and Oyster Bay, Long Island, New York. I entered Graduate College at University of New Hampshire, September, 1927, being enrolled in the Department of Entomology. My minor work was taken in Zoology. I was granted the M.S. degree in June, 1928. Since this time I have been Research Assistant in Entomology at the New Hampshire Agricultural College Experiment Station.

Further graduate study was pursued at Iowa State College in 1932 and 1934-35.

My publications are as follows:


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