# Investigations on Dursban Insecticide. Metabolism of [$^{36}$Cl] *O,O*-Diethyl *O*-3,5,6-Trichloro-2-pyridyl Phosphorothioate in Rats

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When [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl-phosphorothioate is fed as a single dose to rats, the radioactivity is eliminated rapidly via the urine (90%) and feces (10%). The products excreted are [$^{36}$Cl] 3,5,6-trichloro-2-pyridyl phosphate (75 to 80%), [$^{36}$Cl] 3,5,6-trichloro-2-pyridinol (15 to 20%), with traces of *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate. Only the [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate accumulates in the tissues. This accumulates in the fat and is liberated slowly.

The new Dursban insecticide (Dow Chemical Co.) has been found effective in the treatment of parasites in animals. The active ingredient of this insecticide is *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate. As part of the studies on the biological properties of this pyridyl phosphorothioate, a series of metabolism investigations in animals was conducted.

In these investigations the [$^{36}$Cl] chlorine-labeled compound was employed. This was fed to white rats in single oral doses. The rate of elimination of the compound and related metabolites from the animal via the urine and feces was determined. The distribution of radioactive compounds in the tissues was ascertained, together with the rate of elimination of these compounds from the tissues. The possible metabolites of *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate in the tissues and excreta were determined.

In previous studies with this insecticide in plants, it was found that dehalogenation of the [$^{36}$Cl] 3,5,6-trichloro-2-pyridinol could occur and [$^{36}$Cl] chloride could be found in the tissues. To determine the distribution of [$^{36}$Cl] chloride in animal tissues, a similar series of metabolic studies was conducted with sodium [$^{36}$Cl] chloride.

The results obtained in the metabolic investigations with [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate then could be compared with the results obtained in the sodium [$^{36}$Cl] chloride investigations and it would be possible to ascertain if the low levels of radioactivity found in the tissue could be associated with either the [$^{36}$Cl] chloride or the [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate or related compounds.

## *Experimental*

**Chemical.** The [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate was prepared by the direct chlorination of the 6-chloro-2-pyridinol. The synthesis was carried out by the New England Nuclear Corp. based on synthesis procedure developed by Rigterink and Kenaga (*5*). In this procedure, 6-chloro-2-pyridinol was chlorinated with [$^{36}$Cl] hydrochloric acid and hydrogen peroxide. This gave the [$^{36}$Cl] 3,5,6-trichloro-2-pyridinol labeled in the 3 and 5 position. The phosphorothioate was prepared from the 3,5,6-trichloro-2-pyridinol. The radioactive compound had a specific activity of 0.029 mc. per mmole. Infrared analysis and paper chromatography showed that the material had a radiochemical purity of at least 98%. No radioactive compounds other than the *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate could be detected. With both infrared and paper chromatographic procedures, a fraction of a per cent of other radioactive compounds could be present, and they would not be detected by these methods. The phosphorothioate was dissolved in refined corn oil to give a solution containing approximately 10 mg. of the insecticide per ml.

**Treatment of Animals.** A group of white male rats, (Wistar strain about 4 months old) which were selected for uniformity of weight, were placed in individual plastic cages. Each animal weighed approximately 200 grams.

The plastic cages contained a wire floor suspended 2 inches above the bottom of the cage to permit the urine and fecal material to pass rapidly out of the area where the rat was confined. The bottom of the cage was covered with $^1/_2$ inch of sawdust to absorb the urine.

After the animals had been allowed to adjust to their environment of 1 week, each animal was given 1 ml. of the corn oil solution of the [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate. The sample was given by stomach tube to ensure that the complete sample had entered the stomach. The animals were returned to their cages and given free access to food and water. At various time intervals, the animals were sacrificed in groups of two.

The individual animals were anesthetized with Penthane (Abbot Laboratories) and decapitated. The blood was collected with heparin and separated into

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plasma and cells. The individual organs were removed from the animal under conditions which would prevent cross contamination by blood, body fluids, or surgical instruments. The tissues were weighed and frozen immediately. They were kept in the frozen state until analyzed.

A recent series of investigations conducted in our laboratory on the metabolism of [$^{36}$Cl] chlorine-labeled pyridinol compounds in rats, chickens, and dogs showed that there was a minor [$^{36}$Cl] chlorine-labeled material present in trace quantities in the tissues which was eliminated from the body at a rate different from that of the major compound being investigated. Several indications were obtained which suggested that this minor component might be a chloride which was present in the original compound as an impurity, or the compound might be undergoing enzymatic dehalogenation with the formation of radioactive chloride. Because of the small quantities of this minor component in the tissues, it did not appear likely that sufficient material could be isolated to determine its structure by chemical or physical means. An alternate method was therefore used to determine the nature of the minor component found in the tissues of rats fed [$^{36}$Cl] Dursban.

Biological decay studies could be conducted with sodium [$^{36}$Cl] chloride to determine if the distribution of the [$^{36}$Cl] chloride was the same as the minor component present in trace quantities in the tissues, and if the rate of elimination of the [$^{36}$Cl] chloride was the same as the rate of elimination of this minor component.

In the [$^{36}$Cl] chloride experiments each rat was given, via stomach tube, 1 ml. of solution containing 2.32 mg. of sodium [$^{36}$Cl] chloride per ml. with a specific activity of 1 mc. per 232 mg. of sodium $^{36}$Cl chloride. Thus, each animal received 10 $\mu$c. of sodium [$^{36}$Cl] chloride. The rats were placed in metabolism cages and daily collections of urine and feces were made. The samples were analyzed for radioactivity. The animals were sacrificed at various intervals and the organs analyzed for radioactive [$^{36}$Cl] chlorine.

**Method of Analysis.** The amount of radioactivity in each sample was determined by the method of Smith (*6*). This method consists of combusting the tissue in a mixture of 10% nitric acid and 90% sulfuric acid. The chlorine from the compounds was liberated as either chlorine or hydrochloric acid. The chlorine and hydrochloric acid were distilled over into a trapping solution which consisted of 1% arsenious oxide in a 10% solution of sodium hydroxide. The chlorine is converted to chloride by the arsenious oxide and is trapped as sodium chloride. The hydrochloric acid reacted with the sodium hydroxide to form sodium chloride. The chloride ion was precipitated as silver chloride. The silver chloride was collected and washed with diluted nitric acid and acetone to remove any interfering substances. The precipitate was dissolved in 2 ml. of 5% sodium cyanide. The cyanide solution was mixed with 18 ml. of the scintillation solution shown in Table I. The samples were counted in a Nuclear Chicago Scintillation Counter Model 725. Duplicate samples of each tissue were combusted, and the average of the determinations was taken as the value for the individual sample. If the analyses did not agree within a few percentages, additional samples were combusted until satisfactory results were obtained.

**Table I. Scintillation Solution for Counting Silver [$^{36}$Cl] Chloride Dissolved in 2 Ml. of 5% Sodium Cyanide**

| | |
|---|---|
| Dioxane | 500 ml. |
| 2-Ethoxyethanol | 100 ml. |
| Naphthalene | 30 grams |
| PPO | 6 grams |
| POPOP | 0.3 gram |
| "Cab-O-Sil" silica | 18 grams |

*Results and Discussion*

The data obtained by radiochemical analysis of the rat tissues obtained from animals fed [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate are summarized in Table II. In this table, the results are reported in terms of millimoles of radioactive compounds per kilogram of tissue. Radiochemical studies with this insecticide have shown that the compound is metabolized to a wide variety of products. These products all have the same specific activity per millimole of compound, but vary widely in their specific activity

**Table II. Distribution of Radioactivity in Various Tissues of Rats Fed [$^{36}$Cl] *O,O*-Diethyl *O*-3,5,6-Trichloro-2-pyridyl Phosphorothioate**

| Time, Hours | Mmole of Radioactive Compounds per Kg. of Tissue | | | | |
|---|---|---|---|---|---|
| | Liver | Muscle | Heart | Lungs | Spleen |
| 4 | 0.0690 ± 0.0005 | 0.0093 ± 0.0018 | 0.0288 ± 0.0022 | 0.0406 ± 0.0008 | 0.0213 ± 0.0033 |
| 72 | 0.0011 ± 0.0000 | 0.00072 ± 0.00021 | 0.0014 ± 0.0000 | 0.0021 ± 0.0001 | 0.00089 ± 0.00004 |
| 168 | 0.00026 ± 0.00004 | 0.00019 ± 0.00002 | 0.00013 ± 0.00002 | 0.00026 ± 0.00012 | 0.00022 ± 0.00002 |
| 240 | 0.00026 ± 0.00002 | 0.00009 ± 0.00004 | 0.00010 ± 0.00001 | 0.00010 ± 0.00001 | 0.00020 ± 0.00000 |
| 480 | 0.00016 ± 0.00003 | 0.00019 ± 0.00006 | 0.00016 ± 0.00002 | 0.00016 ± 0.00006 | 0.00008 ± 0.00002 |
| | Kidney | Testes | Fat | Bone | Skin |
| 4 | 0.0924 ± 0.0096 | 0.0158 ± 0.0033 | 0.0317 ± 0.0042 | 0.0102 ± 0.0002 | 0.0243 ± 0.0007 |
| 72 | 0.00177 ± 0.00008 | 0.00177 ± 0.00006 | 0.00452 ± 0.00006 | ... | ... |
| 168 | 0.00043 ± 0.00004 | 0.00026 ± 0.00000 | 0.00275 ± 0.00147 | 0.00029 ± 0.00000 | 0.00083 ± 0.00012 |
| 240 | 0.00010 ± 0.00001 | 0.00006 ± 0.00000 | 0.00119 ± 0.00002 | 0.0028 ± 0.00002 | 0.00079 ± 0.00011 |
| 480 | 0.00020 ± 0.00000 | 0.00019 ± 0.00006 | 0.00013 ± 0.00002 | 0.00013 ± 0.00002 | 0.00077 ± 0.00016 |

per gram of compound since they vary considerably in molecular weight. From a radiochemical analysis it is, therefore, possible to compare them on a millimole basis only. The data reported in Table II are typical of results obtained from a labeled phosphorothioate feeding study.

The organs concerned with the elimination of the compound and its metabolites from the body would be high in radioactivity. Both the liver and kidneys were high in radioactivity—i.e., about 0.069 mmole per kg. for liver, and 0.092 mmole per kg. for kidneys.

Phosphorothioates are more soluble in nonpolar solvents, and they should accumulate in the fatty tissues. This was the case with the pyridyl phosphorothioate being studied. High concentrations of radioactivity were found in the fat and skin (about 0.031 mmole per kg. in fat and 0.024 mmole per kg. in the skin). The skin contains a considerable amount of lipid material. The other tissues analyzed varied in their amount of radioactivity. They seemed to vary more or less in relation to the blood content of the sample. For example, the skeletal muscle is lower in radioactivity than the heart or lungs. Once the supply of radioactive compound in the G. I. trace had been exhausted, there was a rapid drop of the level of activity in the other tissues.

By plotting the data on semilogarithmic paper, it was possible to calculate the $t_{1/2}$ value of each tissue. The $t_{1/2}$ value is the biological half-life of the radioactivity in the tissue and is defined as the time necessary to eliminate one half of the radioactive compound or compounds from a given tissue.

Figure 1 shows the curves obtained with kidney, muscle, liver, and fat. In the case of kidney, muscle, and liver, the radioactivity was rapidly eliminated from the tissues. The $t_{1/2}$ value for the radioactive compounds in the liver was approximately 10 hours, while the $t_{1/2}$ value for the compounds in kidney was 12 hours. The compounds in the muscle were eliminated at a slower rate with a $t_{1/2}$ value of 16 hours. The radioactive compounds in the fat had a $t_{1/2}$ value of about 62 hours.

A study of the rate of elimination of the radioactive compounds from the animal via the urine and feces indicated that the majority of the radioactivity appeared in the urine within the first day after the administration of a single dose of the [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (see Table

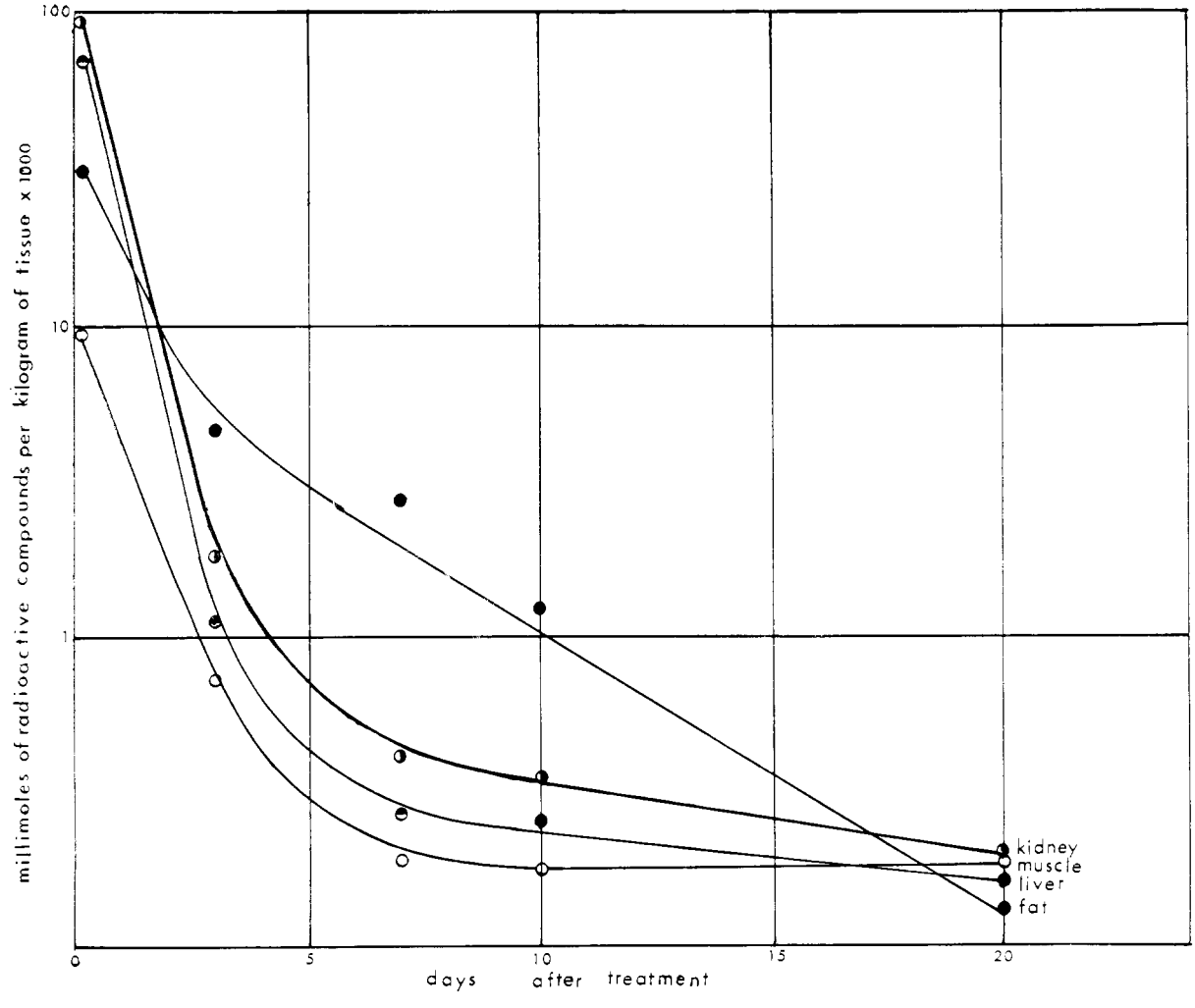


Figure 1. Changes in level of radioactive compounds in rat tissue following single dose of [$^{36}$Cl] Dursban

III). The majority of the activity is eliminated via the urine (90%). This would indicate that the compound is readily absorbed from the G.I. tract and circulated through the body.

**Table III. Elimination of Radioactive Compounds from Rats Following Single Dose of [$^{36}$Cl] *O,O*-Diethyl *O*-3,5,6-Trichloro-2-pyridyl Phosphorothioate (50 Mg./Kg.)**

| Time after Administration of Compound, Hours | Radioactive Compounds Eliminated | | | |
|---|---|---|---|---|
| | Via Urine | | Via Feces | |
| | Mmoles of radioactive compounds per sample | % of total dose (cumulative) | Mmoles of radioactive compounds per sample | % of total dose (cumulative) |
| 18 | 0.0191 | 65.7 | 0.0020 | 6.9 |
| 26 | 0.0044 | 80.8 | 0.0007 | 9.3 |
| 42 | 0.0016 | 86.3 | 0.0003 | 10.3 |
| 50 | 0.0006 | 88.4 | 0.0002 | 11.0 |
| 66 | 0.0002 | 89.1 | 0.0001 | 11.4 |
| 74 | 0.0001 | 89.4 | Trace | 11.4 |
| 90 | Trace | 89.4 | Trace | 11.4 |
| 122 | Trace | 89.4 | Trace | 11.4 |

The results obtained from the studies with sodium [$^{36}$Cl] chloride are summarized in Table IV. In these investigations, the first animals were sacrificed 4 hours after the sodium [$^{36}$Cl] chloride had been given to them. This time interval was selected arbitrarily since by this time the sodium [$^{36}$Cl] chloride administered to the animal would have largely passed from the stomach into the intestinal tract and there should have been maximum absorption of the compound into the circulatory system (*2*). Therefore, the animals were sacrificed at 24-hour intervals. The data given in Table IV were plotted on semilogarithmic paper (see Figures 2 and 3), making it possible to calculate the biological half-life of [$^{36}$Cl] chloride in the various tissues.

In Figure 2, the rate of elimination of [$^{36}$Cl] chloride from the blood, kidney, liver, and muscle is compared. In general, the biological half-life of [$^{36}$Cl] chloride in these tissues was on the order of 40 to 50 hours.

The analysis of the whole blood indicated that there was about twice as much [$^{36}$Cl] chloride in the plasma as in the cells. These results are in agreement with the total chloride found in the blood by chemical analysis. Levitt *et al.* (*3*) reported that plasma from rat blood contains 371 ± 14 mg. of chloride per 100 ml.,

**Table IV. Distribution of [$^{36}$Cl] Chloride in Various Rat Tissues Following Single Oral Dose of Sodium [$^{36}$Cl] Chloride**

| Time, Hours | Mmoles of Radioactive Compounds per Kg. of Tissue | | | | |
|---|---|---|---|---|---|
| | Liver | Muscle | Kidney | Heart | Lungs |
| 4 | 0.0347 ± 0.0039 | 0.0163 ± 0.0028 | 0.0689 ± 0.0017 | 0.0403 ± 0.0030 | 0.0737 ± 0.0062 |
| 24 | 0.0201 ± 0.0001 | 0.0112 ± 0.0006 | 0.0404 ± 0.0042 | 0.0280 ± 0.0053 | 0.0414 ± 0.0021 |
| 48 | 0.0150 ± 0.0004 | 0.0078 ± 0.0013 | 0.0314 ± 0.0047 | 0.0173 ± 0.0042 | 0.0327 ± 0.0028 |
| 96 | 0.0064 ± 0.0006 | 0.0033 ± 0.0006 | 0.0145 ± 0.0007 | 0.0088 ± 0.0030 | 0.0146 ± 0.0018 |
| 168 | 0.0031 ± 0.0003 | 0.0016 ± 0.0001 | 0.0065 ± 0.0004 | 0.0035 ± 0.0002 | 0.0065 ± 0.0001 |
| 240 | 0.0009 ± 0.0004 | 0.0004 ± 0.0001 | 0.0020 ± 0.0010 | 0.0010 ± 0.0002 | 0.0020 ± 0.0010 |
| Biological half life | 40 | 40 | 52 | 40 | 48 |
| Time, Hours | Spleen | Testes | Fat | Stomach | Skin |
| 4 | 0.0481 ± 0.0019 | 0.0662 ± 0.0045 | 0.0278 ± 0.0026 | 0.1520 ± 0.0338 | 0.0491 ± 0.0004 |
| 24 | 0.0305 ± 0.0023 | 0.0470 ± 0.0037 | 0.0107 ± 0.0030 | 0.0659 ± 0.0004 | 0.0371 ± 0.0011 |
| 48 | 0.0232 ± 0.0023 | 0.0383 ± 0.0048 | 0.0071 ± 0.0050 | 0.0465 ± 0.0061 | 0.0249 ± 0.0001 |
| 96 | 0.0100 ± 0.0005 | 0.0160 ± 0.0010 | 0.0074 ± 0.0001 | 0.0163 ± 0.0000 | 0.0122 ± 0.0004 |
| 168 | 0.0044 ± 0.0000 | 0.0065 ± 0.0004 | 0.0029 ± 0.0003 | 0.0061 ± 0.0004 | 0.0025 ± 0.0000 |
| 240 | 0.0014 ± 0.0006 | 0.0021 ± 0.0010 | 0.0006 ± 0.0003 | 0.0025 ± 0.0011 | 0.0020 ± 0.0006 |
| Biological half life | 48 | 52 | 18 | ... | 48 |
| Time, Hours | Bone | Whole Blood | Plasma | Red Cells | |
| 4 | 0.0247 ± 0.0037 | 0.0745 ± 0.0037 | 0.1002 ± 0.0000 | 0.0055 ± 0.0000 | |
| 24 | 0.0176 ± 0.0007 | 0.0528 ± 0.0028 | 0.0602 ± 0.0132 | ... | |
| 48 | 0.0176 ± 0.0025 | 0.0372 ± 0.0047 | 0.0493 ± 0.0069 | 0.0310 ± 0.0039 | |
| 96 | 0.0064 ± 0.0006 | 0.0196 ± 0.0021 | 0.0258 ± 0.0024 | 0.0153 ± 0.0012 | |
| 168 | 0.0028 ± 0.0007 | 0.0080 ± 0.0001 | 0.0105 ± 0.0004 | 0.0064 ● 0.0001 | |
| 240 | 0.0009 ± 0.0004 | 0.0023 ± 0.0009 | 0.0028 ± 0.0016 | 0.0016 ± 0.0006 | |
| Biological half life | 52 | 48 | 48 | 52 | |

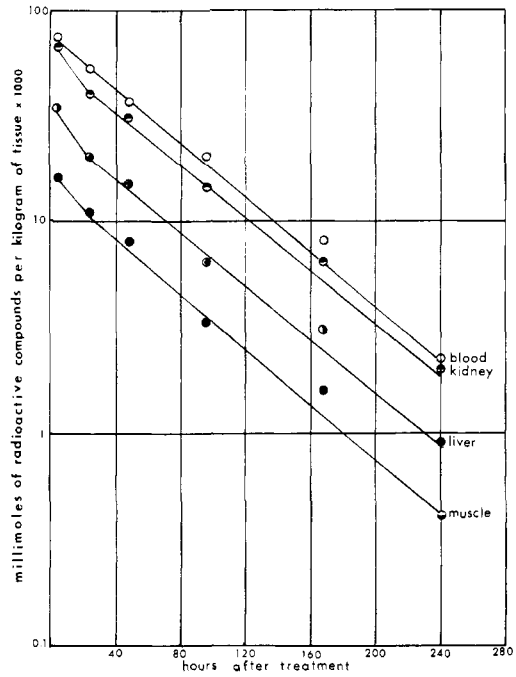


Figure 2. Changes in level of radioactive compounds in rat tissue following single dose of sodium [$^{36}$Cl] chloride

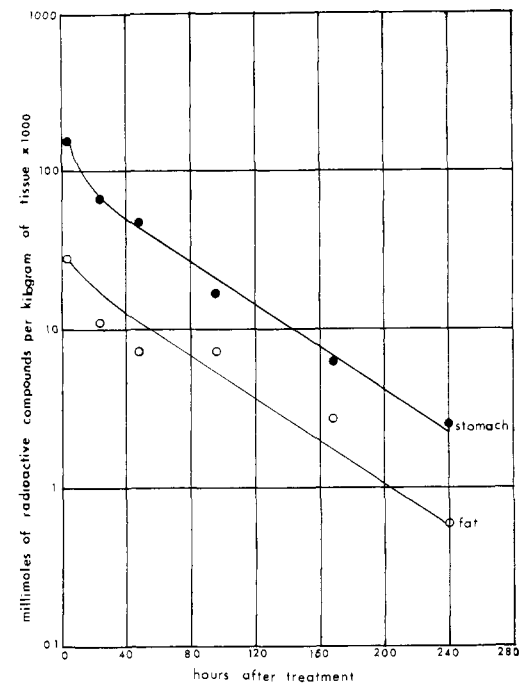


Figure 3. Changes in level of radioactive compounds in rat tissue following single dose of sodium [$^{36}$Cl] chloride

while the cells contain 194 ± 19 mg. of chloride per 100 ml. The biological half-life of [$^{36}$Cl] chloride in blood was 52 hours. The turnover rate in the plasma seemed to be faster than in the cells (48 hours compared with 52 hours).

The biological half life of [$^{36}$Cl] chloride in the kidneys was 52 hours, a little longer than for most of the other tissues. This is not surprising, however, since the kidney would be one of the pathways for the elimination of chloride from the body. The biological half-life of [$^{36}$Cl] chloride in rat liver was 40 hours. This means that there must be a very large turnover of chloride in the rat liver since it has been reported that rat liver contains about 1500 p.p.m. of chloride (*4*). The $t_{1/2}$ value for the [$^{36}$Cl] chloride in muscle was 40 hours, compared with approximately 18 hours for fat.

A comparison of the biological half-lives found with the [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate and sodium [$^{36}$Cl] chloride reveals that, in general, shorter half lives were obtained with the [$^{36}$Cl] phosphorothioate (10 to 16 hours) than with the sodium [$^{36}$Cl] chloride (40 to 50 hours). The noticeable exception to this was in fat. The [$^{36}$Cl] phosphorothioate compounds had a biological half-life of 62 hours in fat compared with 18 hours for [$^{36}$Cl] chloride.

Comparison of the shape of the curves in Figure 1 with those in Figures 2 and 3 shows that several radioactive compounds related to *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate are present in the various organs. The compound with the longest half-life might be [$^{36}$Cl] chloride.

Since most of the tissues lost their radioactivity in a short time after the animal was fed a single dose of [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, there must not be a significant accumulation of [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate or its metabolites in the tissues. The fact that the fat contains a considerable amount of radioactivity which remains for a considerable time makes it desirable to identify the compound present in the fat.

The quantity of radioactive compound in the fat was so low that it precluded the possibility of isolating sufficient quantity of the compound to permit its identification by infrared methods. Therefore, several indirect techniques were used to establish the identity of this material.

Solvent distribution studies showed that if the insecticide and its possible metabolites were distributed between *n*-hexane and 0.1*N* sodium hydroxide solution, the insecticide and its oxygen analog, *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphate, would distribute in favor of the *n*-hexane, while the hydrolyzed products would distribute in favor of the 0.1*N* sodium hydroxide solution. The radioactive material isolated from the fat was distributed in the *n*-hexane. This meant that it was either the insecticide or its oxygen analog.

Enzymatic studies with this insecticide have shown that the *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate is not an inhibitor of fly head cholinesterase, while the oxygen analog is a potent inhibitor. By using the Warburg technique it was found that the radioactive compound in the fat was not a cholinesterase inhibitor. The compound therefore must be the parent compound—i.e., the *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate. This was further confirmed by VPC analysis.

**Table V. $R_f$ Value of Radioactive Compounds Isolated from Urine and Feces of Rats Fed *O,O*-Diethyl *O*-3,5,6-Trichloro-2-pyridyl Phosphorothioate**

| Solvent System[a] | Day | Rat A | | | Rat B | | |
|---|---|---|---|---|---|---|---|
| | | Compd. 1 | Compd. 2 | Compd. 3 | Compd. 1 | Compd. 2 | Compd. 3 |
| | | | | Urine | | | |
| E 3 | 1 | 0.58 | 0.74 | ... | 0.61 | 0.80 | ... |
| E 4 | 1 | 0.59 | 0.76 | ... | 0.62 | 0.82 | ... |
| E 5 | 1 | 0.48 | 0.71 | 0.96 | 0.50 | 0.74 | 0.96 |
| E 3 | 2 | 0.65 | 0.75 | ... | 0.63 | 0.82 | ... |
| E 4 | 2 | 0.61 | 0.78 | ... | 0.61 | 0.76 | ... |
| E 5 | 2 | 0.48 | 0.69 | 0.93 | 0.48 | 0.71 | 0.93 |
| | | | | Feces | | | |
| E 3 | 1 | 0.65 | ... | 0.92 | 0.62 | ... | 0.95 |
| E 4 | 1 | 0.68 | ... | 0.98 | 0.67 | ... | 0.97 |
| E 5 | 1 | 0.46 | ... | 0.97 | 0.48 | ... | 0.97 |

[a] E 3: 40% ethyl alcohol, 40% *n*-propyl alcohol, 5% concentrated ammonium hydroxide, 15% water.
E 4: 75% isopropyl alcohol, 2% concentrated ammonium hydroxide, 23% water.
E 5: 80% acetonitrile, 2% concentrated ammonium hydroxide, 18% water.

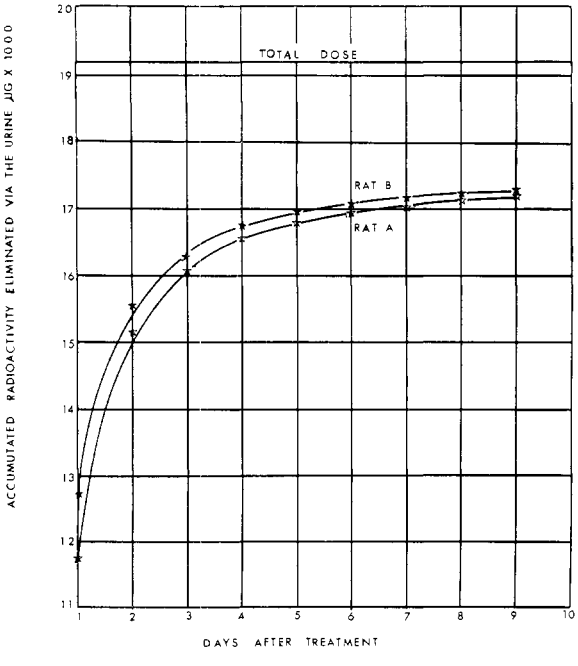


Figure 4. Elimination of radioactive compounds in urine of rats fed single dose of [$^{36}$Cl] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate

**Table VI. $R_f$ Values of Reference Compounds**

| Solvent System[a] | $R_f$ Values | | |
|---|---|---|---|
| | 3,5,6-Trichloro-2-pyridyl phosphate, compd. 1 | 3,5,6-Trichloro-2-pyridinol, compd. 2 | *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, compd. 3 |
| E 3 | 0.59 | 0.76 | 0.94 |
| E 4 | 0.59 | 0.79 | 0.98 |
| E 5 | 0.48 | 0.72 | 0.97 |

[a] E 3, E 4, and E 5 same as in Table V.

**Table VII. Percentage of 3,5,6-Trichloro-2-pyridyl Phosphate (Compd. 1) and 3,5,6-Trichloro-2-pyridinol (Compd. 2) in Rat Urine and Feces**

| Animal | Sample | Post-treatment, Days | Radioactive Compound, % | |
|---|---|---|---|---|
| | | | Compd. 1 | Compd. 2 |
| Animal A | Urine | 1 | 74.1 ± 4.2 | 25.3 ± 4.4 |
| Animal B | Urine | 1 | 81.5 ± 4.2 | 18.0 ± 4.5 |
| Animal A | Urine | 2 | 55.1 ± 3.7 | 44.9 ± 3.7 |
| Animal B | Urine | 2 | 76.4 ± 3.1 | 23.6 ± 3.1 |
| Animal A | Feces | 1 | 86.5 ± 2.4 | 13.5 ± 2.4 |
| Animal B | Feces | 1 | 83.4 ± 3.6 | 16.6 ± 3.2 |

By using a column containing 5% Dow Corning 200 Fluid (12,500 sc) on Chromsorb W at 200° C. a distinct peak was obtained for an authentic sample of *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate. The material obtained from the fat gave an identical peak. This method for VPC identification of *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate is essentially the same as the method of Claborn and Mann which is used for the analysis of the insecticide in animal fat (*1*).

To determine the nature of the radioactive compounds in the urine and feces, two male rats, approximately 200 grams each, were placed in an all-glass metabolism cage and were given approximately 20 mg. of *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate using a stomach tube. Urine and fecal samples were collected daily. Aliquots of each sample were combusted and analyzed for radioactivity.

Urine samples for the 1- and 2-day posttreatment were chromatographed using Whatman No. 1 strips with the descending technique. The strips were developed with the solvent systems shown in Table VI. The developed strips were scanned for radioactivity and radioautographs were made of each strip. On the

basis of the radioautographs and Actigraph scans, each area containing radioactivity was cut from the strip and counted in a scintillation counter.

The radioactivity in the fecal material was extracted with acetone. The acetone extract was then chromatographed.

Figure 4 shows the elimination curves obtained with the animals in the metabolism cage. The urine from the first and second day and the acetone extract of the fecal material from the first day were chromatographed.

The results of the chromatographic studies are shown in Table V using three solvent systems. The radioactive areas were located both by scan and by making radioautographs of the strips. The areas containing the radioactivity were then cut out and counted in a scintillation counter to determine the quantity present in each area. The $R_f$ values of the reference compounds are shown in Table VI.

The data indicate that three compounds are present in the urine and feces: 3,5,6-trichloro-2-pyridyl phosphate (compound 1), 3,5,6-trichloro-2-pyridinol (compound 2), and *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (compound 3). The latter compound is present only in trace quantities. The quantities of compound 1 and 2 were determined by counting the radioactive spots, and the results are shown in Table VII. The results indicate that from 75 to 80% of the radioactivity in the urine is associated with [$^{36}$Cl] 3,5,6-trichloro-2-pyridyl phosphate while the remaining is largely the [$^{36}$Cl] 3,5,6-trichloro-2-pyridinol.

The nature of these two compounds was substantiated further by solvent distribution studies and thin-layer chromatography. With the solvent system *n*-hexane and 0.1*N* sodium hydroxide, the majority of radioactive compounds were found in the sodium hydroxide, indicating that they were hydrolysis products of the insecticide which would form sodium salts. Thin-layer chromatography on Eastman chromatogram sheets gave compounds with an $R_f$ value of 0.18 and 0.00 with *n*-hexane, acetone, and concentrated ammonium hydroxide (80:18:2) and 0.75 and 0.31 with acetone, methyl alcohol, and concentrated ammonium hydroxide (93:3:2). These $R_f$ values correspond to those obtained with the 3,5,6-trichloro-2-pyridinol and 3,5,6-trichloro-2-pyridyl phosphate.

*Literature Cited*


(1) Claborn, H. V., Mann, H. D., Pesticide Chemical Research Branch, Entomology Division, Agricultural Research Service, U.S.D.A., Kerrville, Tex., personal communication.
(2) Feen, W. O., *J. Biol. Chem.* **128,** 297 (1939).
(3) Levitt, M. F., Turner, L. B., Sweet, A. Y., Pandiri, Demetra, *J. Clin. Invest.* **35,** 98 (1956).
(4) Long, C., King, E. J., Sperry, W. M., "Biochemist's Handbook," Van Nostrand, London, 1961.
(5) Rigterink, R. H., Kenaga, E. E., J. AGR. FOOD CHEM. **14,** 304 (1966).
(6) Smith, G. N., *J. Anal. Biochem.* **17,** 24 (1966).



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