

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Bird Control Seminars Proceedings

Wildlife Damage Management, Internet Center
for

September 1970

NONBIOLOGICAL ALTERATION OF 3-CHLORO P-TOLUIDINE HYDROCHLORIDE (DRC-1339) IN AN AQUEOUS SOLUTION

T. R. Darden

University of California, Davis

Robert G. Schwab

University of California, Davis

Follow this and additional works at: <https://digitalcommons.unl.edu/icwdmbirdcontrol>



Part of the [Environmental Sciences Commons](#)

Darden, T. R. and Schwab, Robert G., "NONBIOLOGICAL ALTERATION OF 3-CHLORO P-TOLUIDINE HYDROCHLORIDE (DRC-1339) IN AN AQUEOUS SOLUTION" (1970). *Bird Control Seminars Proceedings*. 197.

<https://digitalcommons.unl.edu/icwdmbirdcontrol/197>

This Article is brought to you for free and open access by the Wildlife Damage Management, Internet Center for at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Bird Control Seminars Proceedings by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

NONBIOLOGICAL ALTERATION OF 3-CHLORO P-TOLUIDINE HYDROCHLORIDE (DRC-1339) IN AN AQUEOUS SOLUTION

T. R. Darden and R. G. Schwab
Department of Animal Physiology
University of California
Davis, California

The effectiveness of 3-chloro p-toluidine (DRC-1339) as an avicide, particularly on the European starling, has been adequately demonstrated (DeCino, Cunningham, and Schafer, 1966; Schwab, 1965, 1966, and 1967). Methods of applying the compound which have proved useful range from the standard baiting technique (Besser, Royall, and DeGrazio, 1967; Royall, DeCino, and Besser, 1967; West, 1968) to possible aerial application of the compound to roosting sites (Schwab, 1967). These methods, particularly the latter, inherently lead to widespread environmental exposure. As the chemical name implies, DRC-1339 is a chlorinated hydrocarbon. It thereby carries the stigma, whether justifiable or not, of environmental persistence and concentration at the upper end of the food chain (Rudd, 1966). Therefore, before the compound can be used as a widespread agent in bird control, its persistence and physical behavior in the ecosystem must be determined.

Unlike many chlorinated hydrocarbons, 3-chloro p-toluidine is water soluble within the range of biological effectiveness as an avicide. As a first step then, we have tested its stability in water when exposed to the common physical components of the environment. The effects of heat, cold, light, oxidation, and evaporation have been tested.

GENERAL METHODS

Two methods of assaying the concentration (activity) of 1339 have been used throughout this study. The first is the spectrophotometric analysis or chemical test based on the Bratton-Marshall reaction for primary aryl amines as modified by Peoples (1967). These chemical tests were carried out during the various experiments in order to ascertain both extent and the time course of change. Biological assays were performed at the end of most tests to insure biological reliability of the chemical tests. All bioassays were performed on first year female starlings by intubing 0.25 to 0.75 cc of solution directly into the G.I. tract of the bird. Dosages for bioassays were calculated on the basis of the chemical test results using the standard mg/kg method. The time of intubation was kept within three hours after sunrise in order to minimize the effects of the well documented circadian susceptibility rhythm present in starlings (Schwab, Eglehoffer, and Osborne, 1967). Periodic LD-50 determinations were performed throughout the period of testing using the untreated compound. These tests served as controls.

DESCRIPTION OF TESTS AND RESULTS

Stability of 1339 in water: oxidation.

The stability of two different concentrations of 1339 in water was measured over a period of several months. The solutions were maintained in two 500 ml graduated cylinders. Oxidation was speeded up by having O₂ slowly bubble through each solution. Volatilization was presumably suppressed by a filter paper placed over the tops of the cylinders. A detailed discussion of this experiment will be reported elsewhere. The results can be summarized briefly (Dr. Alex Apostolou, personal communication). Solutions of 1339 turned yellow in time, the extent of coloration depended upon time exposed and the initial concentration of 1339. This process ultimately led to formation of a yellow precipitate of unknown composition. The toxicity of the precipitate is questionable. After 5 months exposure, total loss of compound, *i.e.*, total remaining in solution, plus dried weight of the precipitate, minus initial concentration, was small-averaging 6%. The results indicate that under these conditions 1339 is a very stable compound which oxidizes slowly and to minor degree.

Effects of cold treatment.

Solutions of 1339 (1 mg/kg) were frozen for various periods of time. Samples were taken both before and after freezing and tested chemically. An additional solution was frozen, thawed and refrozen five times and tested chemically and biologically at the end of exposure. In neither experiment was a significant difference in either chemical or biological activity noticed. Refer to Table 1 for a detailed listing of the results. The bioassay results were obtained by intubing ten birds with a 2.5 mg/kg solution; six birds were killed.

Effects of heat treatment.

The effects of heat on 1339 activity were examined using three different exposure temperatures: 50°C, 75°C, and 100°C. The first temperature is a commonly observed leaf and soil surface figure; the other two higher temperatures were chosen as a test of extremes. Ten milliliters of a 5 mg/ml solution of 1339 were placed in a 10 ml graduated cylinder which in turn was placed in a temperature controlled oil bath. Samples (0.1 ml) were extracted periodically and tested chemically. The volume of the cylinder was recorded at each sampling interval and the total concentration of 1339 calculated. Each test was repeated twice at all three temperatures. The graphical representation of the results appears in Figure 1.

At all three temperatures the total concentration of 1339 decreased. At all three levels a progressive yellowing of the solution occurred, becoming very marked after 6 hours in the 100°C bath and requiring 16-42 hours in the 50°C bath. In addition, the aforementioned precipitate formed at all three levels. This precipitate was separated at

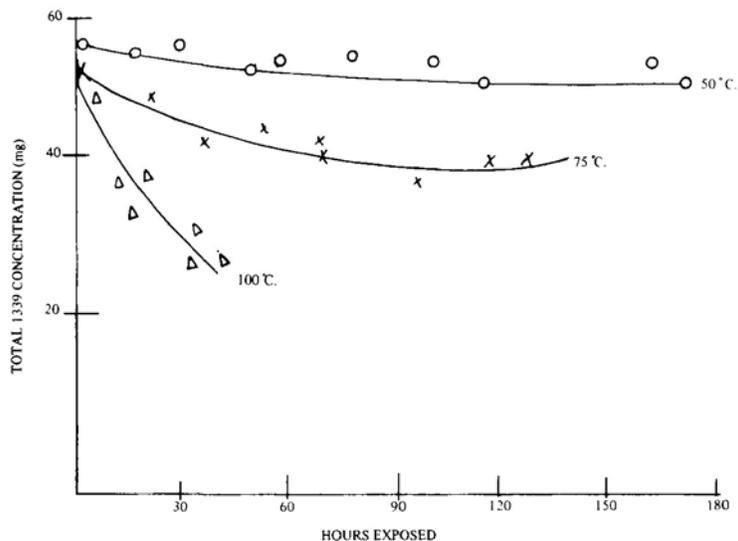


Figure 1. The three curves represent the change in total 1339 concentration (as determined chemically) at three different temperatures through time. Total exposure time differs due to more rapid evaporation of the solvent at the higher temperature.

the end of one test at both the 50°C and 75°C level. The solid material was dried at room temperature and weighed. After 95 hours of exposure at 75°C the total weight of the solid material (7.5mg) accounted for 35% of the total amount of 1339 which had disappeared from solution (22mg). After 166 hours of exposure at 50°C, the precipitate (2.5mg) accounted for 29% of the total (9mg) lost from solution.

Bioassays based on the 1339 concentration chemically determined at the end of one 50°C and 75°C test were performed. Dosages of 3.1mg/kg were given to two groups of ten birds each. This dose lies between the LD-50 and LD-100 for this compound (see Figure 2). No significant difference in percent killed with treated and untreated material was found. Seven of ten birds died in the 75°C group and nine of ten died in the 50°C group.

The above results indicate that some alteration of the solutions of 1339 does occur in the presence of high temperatures. In part (about 30% of the amount lost) this is due to the oxidation reaction which caused the precipitate to form and thus removed some of the compound from solution. The remaining difference cannot be accounted for at this time. Whether the compound is detoxified and remains in solution or volatilizes in its unaltered form is unknown.

Volatilization.

The fact that some chlorinated hydrocarbon solutions are slightly volatile is considered a possible mechanism which may account for the present world-wide distribution of DDT, DDE, and other organochlorine pesticides (Tatton and Ruzicka,

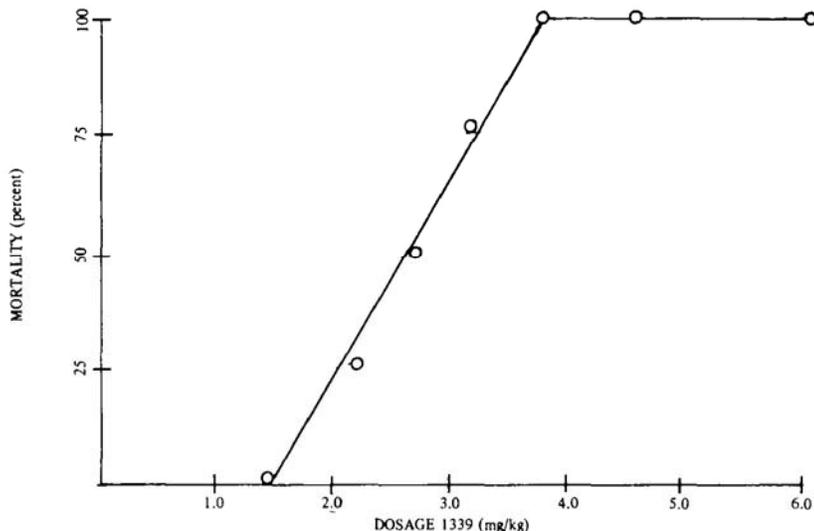


Figure 2. Typical L.D.-50 curve established during the testing period. This curve was determined between 13 and 16 December 1969 at 8:30 a.m. on first year female birds (n=38). Calculation of L.S.-50 by Weil method = 2.48 mg/kg with 95% confidence limits of 2.42-2.54 mg/kg.

1967). The following experiment was performed to obtain a preliminary determination of the extent to which a 1339 solution volatilizes. In order to distinguish between loss of compound by volatilization or oxidation, the test solution was made in 1.2N HCl. Such solutions, if kept in stoppered containers are stable for long periods of time and serve as standards in spectrophotometric analysis. Specifically, a 10 gamma/ml 1339 solution in 1.2N HCl was prepared in early October 1969 and utilized for standard analysis until May 1970. No significant change in 1339 concentration as measured by absorbance (Bausch and Lomb-Spectronic 20) occurred during this interval. Seventy-two absorbance readings taken periodically ranged from 0.57 to 0.72 O.D. with a mean of 0.63 ± 0.02 (S.E.). Further, acid solutions of 1339 do not oxidize and thus do not become yellow. Therefore, 1339 in HCl solutions is not subject to an appreciable amount of oxidation, and thus, the degree of volatilization of this compound can be measured from the acid system.

A solution containing 12 mg of DRC-1339 in 1 liter of 1.2N HCl was placed in a graduated cylinder. Except for removal of samples via pipette, this solution remained undisturbed, shielded from ultra violet light, and at room temperature (about 22°C) for about four months. The surface area of solution exposed to the atmosphere was about 5 in².

Periodically, samples were taken from this solution and chemically analyzed for 1339 concentration (Table 2). DRC-1339 concentrations in five samples taken periodically between 23 January and 1 April indicated a progressive decline as a function of exposure time. After 66 days exposure, 24 percent of the 1339 was lost from the solution, presumably as a result of volatilization.

Table 1. Results of chemical and biological assays on solutions exposed to freezing temperatures.

<u>Test No.</u>	<u>Explanation of test</u>	<u>Original concentration</u>	<u>Concentration following test</u>	<u>% change</u>	<u>Bioassay % kill</u>
1	Sol. frozen 3 days	1.0 mg/ml	0.98 mg/ml	-2%	---
2	Sol. frozen 7 days	1.0 mg/ml	0.97 mg/ml	-3%	---
3	Sol. frozen 11 days	1.0 mg/ml	1.07 mg/ml	+7%	---
4	Sol. frozen 20 days	1.0 mg/ml	1.00 mg/ml	00	---
5	Sol. frozen thawed and refrozen during 30 days period.	1.0 mg/ml	0.99 mg/ml	-1%	60%**

** Refer to Fig. 2 for control bioassay data.

Table 2. Volatilization of 1339 from an acid solution

<u>Sample No.</u>	<u>Date</u>	<u>Days Exposed</u>	<u>Volume of Solution</u>	<u>Total Concentration (gammas=mg x 10³)</u>	<u>% 1339 Lost</u>
1	23 Jan	0	1000	12,000	0
2	31 Jan	8	960	11,040	8
3	17 Feb	25	885	11,000	9
4	13 Mar	48	790	10,010	15
5	1 Apr	66	710	9,100	24
6	14 May	110	1000	9,500	21

After 110 days of exposure, the solution was brought to its initial volume by addition of 1.2N HCl. The solution was then thoroughly stirred to recover adhered material and the concentration of DRC-1339 again determined. Slightly more of the compound was detected in this final sample due presumably to recovery of material adhered to the wall of the cylinder. After 110 days exposure, 2.5 mg was lost via volatilization from an initial total of 12 mg. This is an appreciable amount considering that the area of solution exposed to the atmosphere was only 5 in².

It seems reasonable to assume that a decided increase in the rate of volatilization would occur from a thinly applied spray (such as would be applied via airplane to treat a roost area) because of the increased exposure area. What chemical alterations this organochloride undergoes in the upper atmosphere becomes a crucial question.

Light.

It has been suggested that ultraviolet light might detoxify 1339. Recently, the U.S. Fish and Wildlife Service reported that a UV light absorber placed in a 1339 derivative (DRC-1347) extended its field effectiveness (Schafer, West, and Cunningham, 1969). To distinguish between the effects of volatilization and UV light and slow oxidation 1339 solutions were sealed within quartz or plain glass tubes. Experiments were divided into two groups; first, experiments conducted using a UV lamp of high intensity and short wave length and second, experiments conducted using direct sunlight. The lamp used in the first part was a "Mineral Light" model UVS-11 (Ultra-Violet Products) with a peak output of 2537 Å and intensity of 80 $\mu\text{W}/\text{cm}^2$. Tests were conducted with the lamp 3" away from the tube. Quartz is penetrable to more than 80% of the incident UV radiation (Hodgman, 1962).

Artificial UV source.

Two different tests involving continuous exposure and periodic sampling, were carried out with the UV lamp. Figure 3 shows the rate and extent of deterioration based on chemical analysis. In all cases, the control solution was sealed in an identical quartz tube and exposed to room lighting. The rate of chemical change in the two tests (Figure 3) is, for all practical purposes, the same.

The color change of the treated solution was typical of that described above during the early portion of exposure. Due to the small amount of air which came in contact with the solution, the extent of this color change was limited. However, after 150-200 hours of exposure the solution turned a dark chocolate-brown color which was not observed in the control tubes and was unaccompanied by the formation of a precipitate. The lack of precipitate also holds for the control and probably results from a minimum amount of oxidation within the sealed tubes.

The brown solution (from the initial 5mg/ml solution) which according to chemical assay contained 1.1 mg/ml (Figure 3) was used for bioassay. Four groups of starlings (4 birds/group) were intubed with the treated solution in the following concentrations: 3.4, 4.1, 4.9, and 6.9 mg/kg. The first three dosages were calculated

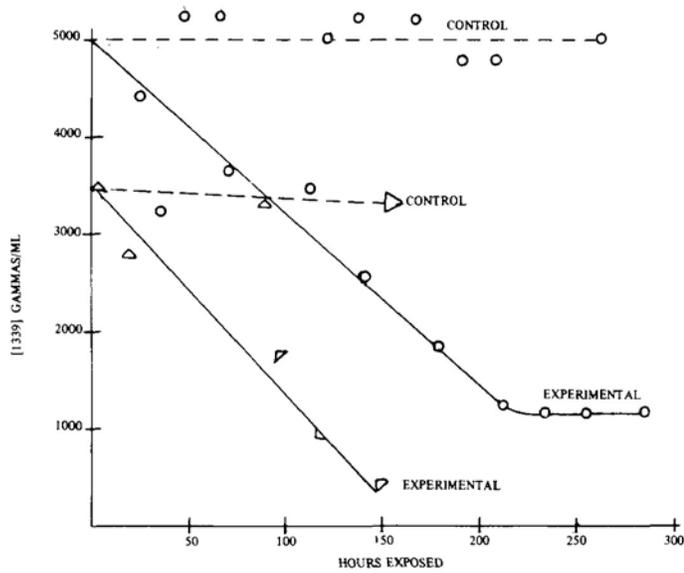


Figure 3. Extent and time required to degrade an aqueous solution of 1339 using U.V. light of high intensity and short wave length. See text for details.

on a ratio basis (1.2 to 1), the fourth used the remaining compound given full strength. In addition, two other groups of four birds were intubated using the control solution at dosages of 3.4 and 4.1 mg/kg. The results (Figure 4) of the bioassay and chemical assay for the treated solution are distinctly different. Therefore, the exposure to high intensity short wave UV light can cause a chemical change which is unlike that seen in the oxidation or heating tests and the full extent of which is undetectable with standard chemical analysis.

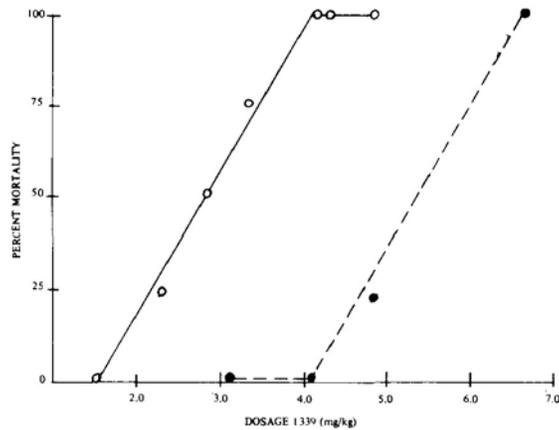


Figure 4. Dose response curve for solution treated with ultraviolet light. Solid circles and broken line represent treated solution, open circles and solid line = values for untreated solution transposed from Figure 2.

Ultraviolet light of this wave length and intensity causes a chemical and biological activity change. The rate of breakdown varies directly with intensity and time (with wave length held constant). In this artificial lab system, the extent of breakdown may be altered by the penetrability of the solution to UV light. Light penetrability decreases as the solution becomes dark brown and may explain the plateau reached in Figure 3 (5 mg solution). The 3 mg solution did not reach a decay plateau, but neither did it turn as dark brown as the stronger solution.

The wave length of UV light used in these tests does not reach the earth's surface since it is filtered out by the Ozone layer in the stratosphere but is present at the 35 Km level and above (Green, 1966).

Natural UV source.

To establish the rate of change of 1339 solution exposed to sunlight, three similar tests were performed outside where UV light of longer wave lengths (> 3000 Å) occur. In all cases the control solution was placed in a plain glass tube (impenetrable to UV light); the test solution in a quartz tube. Intensity of solar UV light varies greatly with atmospheric conditions, and is therefore difficult to quantify. The first test was conducted during mid-winter with 12 days of total overcast, the second, shortly thereafter, under partly cloudy skies, the third lasted eight days, all of them very clear. Figure 4 depicts the results of all three experiments, no significant difference exists between the tests, and the data were pooled. The effect of natural radiation on 1339 solutions is minor for the time exposed. From day nine through day fourteen (90-140 hours), the concentration began to show a slight decline and regression lines established from the control and experimental data have statistically different slopes ($p < 10$), (figure 5).

The solution from the third test was used for a bioassay and prepared based upon chemical assay to 3.1 mg/kg. Eight birds were intubed with the solution; seven birds died all showing typical 1339 poisoning symptoms as described by Peoples and Apostolou (1966). This figure (88%) is well within the expected percent kill for an untreated solution. Unlike the artificial short wave, high intensity ultraviolet light, natural sunlight appears less effective in destroying the activity of DRC-1339. However, the poor climatic conditions existing during two of the tests may have altered the final result. In addition the longer, but lower energy UV light, spectrum reaching the earth's surface exhibits low penetrability thus compounding the effects previously discussed that would limit decomposition. When the free base of 3-chloro p-toluidine (DRC-1347) was exposed to sunlight in thin layers, its toxicity to starlings began to decline within two days (Schafer, West, and Cunningham, 1969). Therefore, there remains little reason to doubt that DRC-1339 would decompose to a greater extent than shown in the above experimental situation if exposed to sunlight as a thin, unprotected layer.

Combined effects.

The previous tests were designed to examine nonbiological factors individually as they affect the degradation of 1339. The next two experiments were performed

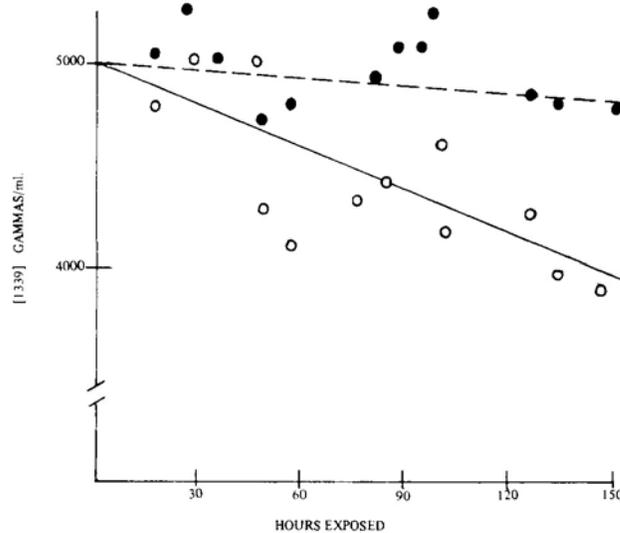


Figure 5. Results of exposure of 1339 solutions to sunlight. An average value of 10 possible hours of exposure to direct sunlight per day was used throughout the experiments. Open circles are experimental values, solid circles are controls. Regression lines established by least squares analysis.

to obtain an estimate of the combined effects of UV light, heat, and volatilization on the disappearance of 1339. In the first test, a 4 mg/ml solution of 1339 was prepared. One hundred lambdas of this solution were pipetted onto 40 clean glass slides. Four slides were immediately submerged in 50.0 ml of distilled water contained in slide jars. This was done to remove the adhered compound from the slides at the same time diluting the sample for spectrophotometric analysis. The remainder of the slides were exposed to the direct sunlight from 9 a.m. to 5 p.m. (August, clear day). Every hour four slides were removed from exposure and placed to soak in 50.0 ml of distilled water. The slide surface temperature was also recorded at this time. The slide jars were covered and stored in a dark cabinet for 4 days. The 1339 concentration was then determined chemically. The results of this first test are depicted in Figure 6.

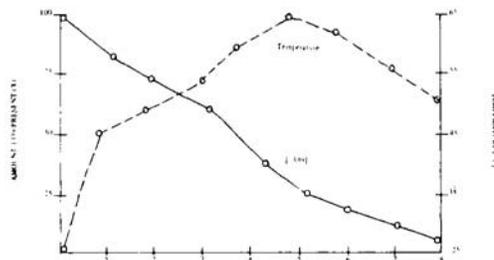


Figure 6. Combined effects of U.V. light, heat and volatilization on the disappearance of 1339 when exposed as a thin liquid film.

The data are presented in terms of percent disappearance of the compound. Eight hours of exposure of a thin layer of 1339 to the mid-summer elements in Davis causes an almost complete disappearance of the compound. The rate of extinction is linear during the first and last thirds of exposure time. The rate shows a striking correlation with the most rapid increase in temperature following initial warm-up of the slides (hours 3-5). The liquid on all slides had evaporated to dryness after one hour.

The results of this test are clear cut-light, heat, and volatilization working together can cause very rapid disappearance of the compound from a thinly applied layer. Recovery of the compound from the slide surface is not a factor since the disappearance was definitely linear, thus precluding any constant adhesion. Since the liquid was completely gone within one hour, the physical factors are operating on the thin powdery residue of the pure compound during most of the exposure time. The correlation of rapidly increasing temperature with rapidly decreasing 1339 presence may be due to either increased UV intensity or increased temperature or both.

The second test was performed exactly as the first, but the slides were placed in a refrigerator, thus eliminating the influence of heat, UV light, and slowing volatilization. The results of this test are shown in Figure 7. The results obtained for hour six were aberrant and are not included. The liquid evaporated from all slides by the sixth hour.

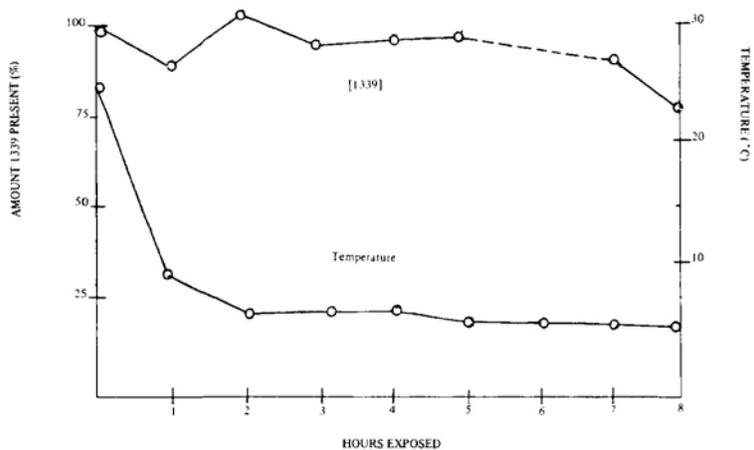


Figure 7. Effects of exposure of a thin queous layer of 1339 to low temperature, slow volatilization and darkness. The sixth hour sample was aberrant and thus is not shown.

It has previously been shown that low temperatures alone do not affect the activity of 1339. The initial 10% loss during the first 5 hours may best be attributed to volatilization. The remaining 20% loss occurring during the last two hours may perhaps be due to poor recovery of the compound from the slides. However, the decrease during those last hours appears linear indicating that other factors may be operating. It may be possible that volatilization from the solid state occurred. Further investigation of this point seems warranted.

No source of UV light or heat was present during the second test. Volatilization from both the liquid and solid phases is the only obvious pathway which can explain the disappearance. The rate of volatilization increases with increasing temperature and thus, it is likely that more than 30% of compound loss in the first test can be attributed to volatilization.

DISCUSSION AND SUMMARY

The persistence of some organochloride pesticides (and their metabolites) has led to both the banning and restricting of their use by governmental agencies. The most common substitutes for these pesticides are highly toxic but short-lived chemical control agents. The demand now is for specific nonpersistent pesticides.

The toxicity of DRC-1339 to mammals, administered both acutely and chronically, is reduced by a factor of 2 to 3 below that for birds (Peoples, 1965). Limited tests on secondary hazards also indicate the safety of the compound (Henry, Campney, and Cummings, 1964). The toxicity of this avicide and its metabolites to invertebrate populations is currently under investigation. Thus far, all data strongly support the claimed specificity for DRC-1339.

The present investigation strongly indicates that physical environmental characteristics can reduce the biological activity of 3-chloro p-toluidine hydrochloride when acting in concert on a thin liquid or solid layer. These factors—light, heat, and volatilization—cause the complete disappearance of DRC-1339 within a few hours.

Two distinct phenomena have been observed during these tests, and great emphasis must be placed on their differences. Chemical alteration which led to decreased biological toxicity was observed in the short wave UV "light" test. Thus, a difference between chemical and biological activity can be created by short wave UV light. Although this particular light spectrum doesn't penetrate to the earth's surface it is present in the stratosphere (Green, 1966). Thus, volatilized DRC-1339 carried into the upper atmosphere may be rapidly detoxified. The other type of chemical alteration observed is the oxidation product (yellow precipitate) isolated during the "water stability" and "heat" tests. The structure and toxicity of this compound is presently unknown.

The second phenomena observed is the apparent disappearance of DRC-1339. In both the "heat" and "volatilization" tests, recovery of the compound was not complete. Therefore, the avicide became either chemically undetectable, and presumably biologically inactive, or was lost from the system through volatilization. The latter pathway for disappearance is the most probable but can lead to false assumptions about apparent degradation from an aerial spray target area and disperse the still toxic material into the surrounding environment. The results of the light test indicate strongly however, that detoxification will be rapid once the vapor is exposed to light.

Thus, all indications point to the fact that DRC-1339 fits the current requirements for a chemical control agent, i.e., short environmental half life and species specificity.

LITERATURE CITED

- Besser, J. F., W. C. Royall, Jr., and J. W. DeGrazio. 1967. Baiting starlings with DRC-1339 at a cattle feedlot. *J. Wildl. Manage.* 31:48-51
- DeCino, T. J., D. J. Cunningham, and E. W. Schafer, Jr. 1966. Toxicity of DRC-1339 to starlings. *J. Wildl. Manage.* 30:249-253
- Green, A. E. S. 1966. *The Middle Ultra Violet: Its Science and Technology.* John Wiley & Sons. New York
- Henry, J., M. Campney and M. Cummings. 1964. Secondary toxicity hazard of DRC-1339. *In Progress report on starling control.* R. G. Schwab [ed.] Univ. Calif. Agric. Exp. Sta. p. 16.
- Hodgman, C. D. 1962, (Editor) *Handbook of Chemistry and Physics.* 44th ed. Chemical Rubber Publ. Co., Cleveland.
- Peoples, S. A. 1965. Studies on the use of DRC-1339. *In Progress report on starling control.* R. G. Schwab [ed.] Univ. Calif. Agric. Exp. Sta. p. 12-19.
- Peoples, S. A. and A. Apostolou. 1966. Studies on the mechanism of the toxic action of 3-chloro p-toluidine (DRC-1339) on starlings. *In Progress report on starling control.* R. G. Schwab [ed.] Univ. Calif. Agric. Exp. Sta. p. 13-14
- Peoples, S. A. 1967. A colorimetric method for DRC-1339 and its metabolites in bird feces and mammalian urine. *In Progress report on starling control.* R. G. Schwab [ed.] Univ. Calif. Agric. Exp. Sta. p. 10-11.
- Royall, W. C, Jr., T. J. DeCino, and J. F. Besser. 1967. Reduction of a starling population at a turkey farm. *Poul. Sci.* 46:1494-1495.
- Rudd, R. L. 1966. *Pesticides and the Living Landscape.* Univ. Wisc. Press, Madison.
- Schafer, E. W., R. R. West, and D. J. Cunningham. 1969. New starling toxicant: DRC-1347. *Pest Control.* 37:22-30.
- Schwab, R. G. 1965. Preparation of DRC-1339 treated raisins. *In Progress report on starling control.* C. C. Seibe, A. Zajanc, and R. Schwab [ed.] Univ. Calif. Agric. Exp. Sta. p. 27-30.
- Schwab, R. G. 1966. (Editor) *Progress report on starling control.* Univ. Calif. Agric. Exp. Sta.
- Schwab, R. G. 1967. (Editor) *Progress report on starling control.* Univ. Calif. Agric. Exp. Sta.

Schwab, R. G. and Eglehoffer, S. 1967. DRC-1339 as a contact toxicant for starling control. *In* Progress report on starling control. R. G. Schwab [ed.] Univ. Calif. Agric. Exp. Sta. p. 14-20.

Schwab, R. G., S. Eglehoffer, and J. Osborne. 1967. Daily and seasonal susceptibility of starlings to the avicide DRC-1339. *In* Progress report on starling control. R. G. Schwab [ed.] Univ. Calif. Agric. Exp. Sta. p. 20-37.

Tatton, J. O'G., and J. H. A. Ruzicka. 1967. Organochlorine pesticides in Anarctica. *Nature*. 215:346-348.

West, R. R. 1968. Reduction of a winter starling population by baiting its pre-roosting areas. *J. Wildl. Manage.* 32:637-640.